ESTIMATING GAINS FROM GENETIC TESTS OF SOMATIC EMBLINGS OF INTERIOR SPRUCE

G.A. O'Neill [1], J.H. Russell [2], B.D. Hooge [3], P.K. Ott [4] & C.B.D. Hawkins [5]

[1)] British Columbia Ministry of Forests, Research Branch, Kalamalka Forestry Centre, 3401 Reservoir Road, Vernon, BC, Canada V1B 2C7. 250-260-4776 (Tel); 250-542-2230 (fax) greg.oneill@gems6.gov.bc.ca
[2)] British Columbia Ministry of Forests, Research Branch, Cowichan Lake Research Station, Mesachie Lake, BC, Canada V0R 2N0. 250-749-6811 (Tel); 250-749-6020 (fax) john.russell@gems1.gov.bc.ca
[3)] British Columbia Ministry of Forests, Research Branch, Prince George Tree Improvement Station, 17000 Domano Blvd. Prince George, BC, Canada V2N 5M2. 250-963-8416 (Tel); (250) 963-3116 (fax) Bonnie.Hooge@gems6.gov.bc.ca
[4)] British Columbia Ministry of Forests, Research Branch, 1st Floor, 722 Johnson St., Victoria, BC, Canada V8W 1N1. 250 387-7982 (Tel); 250 387-0046 (fax) Peter.Ott@gems7.gov.bc.ca
[5)] Mixwoods, Ecosystem Science and Management Program, University of Northern British Columbia, 3333 University Way, Prince George, BC, Canada V2N 4Z9. 250-960-5614 (Tel); 250-960-5539 (Fax) hawkinsc@unbc.ca
[1)] corresponding author

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ABSTRACT

To assess the growth of somatic emblings *versus* seedlings and to estimate genetic parameters and gain that would accrue from a clonal testing and selection program, seedlings and 313 embling clones from 11 full-sib families of interior spruce (*Picea glauca* (Moench) Voss and *P. engelmannii* Parry and their hybrid complex) were assessed at two test sites in British Columbia.

Seedlings were 11% taller than emblings after 7 years, however, when height at planting (age-one) was accounted for, the difference between the two stocktypes was reduced to 8 % (p = 0.0414). Height-age trajectories between stocktypes were similar. Repeatabilities for height at age-7 were strong for clones (h^2_C = 0.71; $h^2_{C(F)}$ = 0.69) and families (h^2_F = 0.80). Variation was relatively high among clones (CV_C % = 14.6 %; $CV_{C(F)}$ % = 13.3 %), but low among families (CV_F % = 5.9). Genetic gains in excess of the seed orchard alternative were estimated for selection scenarios involving hypothetical tests containing between 200 and 2000 clones from 40 families. Gain for direct selection for height at age-7 was large: 21.5 % for mass clonal selection if the tallest 20 of 200 clones were selected, increasing to 32.8 % if 20 of 2000 clones were selected. Substantial relatedness among the tallest clones was avoided by using 2-stage (family and clone-within-family) selection, however, gains were about 4 % less (*e.g.*, 17.0 % and 28.9 % for tests of 200 and 2000 clones respectively) than for unrestricted (mass clonal) selection. Gain estimates are contingent on improved propagation methods being able to produce seedlings and emblings that possess equivalent growth rates. These results indicate that testing and deployment of embling clones can produce significant gains beyond that expected from seed orchards, however several technical and economic issues remain to be addressed.

Keywords: *Picea glauca*, *Picea engelmannii*, somatic embryogenesis, genetic gain, clonal testing, emblings

INTRODUCTION

The advantages of clonal forestry (testing, selection and deployment of elite clones) over a conventional sexual-based recurrent selection program in tree improvement have been well-documented (LIBBY & RAUTER 1984). Some of the advantages include greater gain due to capture of additional genetic variance, increased uniformity among propagules, and earlier deployment of elite genotypes. However, traditional methods of vegetative propagation such as rooting of cuttings are used in only a few species due to expense, poor field performance, or maturation-related problems that hamper rooting success and reduce the quality of the propagules (TALBERT *et al.* 1993).

Somatic embryogenesis (SE) is a relatively new form of tissue culture that has been used extensively in horticulture and agriculture to rapidly multiply selected varieties or genotypes. Embryos of selected individuals are excised from seeds and induced to form embryogenic cultures, in which each new embryo is genetically identical to the original embryo. The term 'somatic' refers to embryos develop-

ing asexually from vegetative (somatic) tissue. New embryos are germinated and transplanted in containerized nursery operations (WEBSTER *et al.* 1990).

The main advantage of somatic embryogenesis over other forms of vegetative propagation is that it circumvents problems related to maturation of ortets that can impair rooting and subsequent growth of propagules. Cryopreservation of embryogenic cultures during genetic testing permits returning to the original cultures once elite individuals (also called lines, clones or genotypes) are identified. Embryogenic cultures of the elite individuals can then be thawed and mass-propagated. In addition, the procedure assures minimal stem plagiotropism, and bulking-up can potentially be faster than other forms of vegetative propagation (GROSSNICKLE *et al.* 1996; HÖGBERG *et al.* 1998).

Interior spruce (*Picea glauca* (Moench) Voss and *P. engelmannii* Parry and their hybrid complex) tree improvement in British Columbia (BC) is in the second generation of a recurrent selection program for height growth and weevil resistance. Rogued first generation spruce seed orchards are currently producing 95 % of the province's spruce seed demand in eight of nine spruce breeding zones, providing an average of 12 % genetic gain in volume in those zones (pers. comm., B. Jaquish, Research Branch, British Columbia Ministry of Forests). Elite parents for height growth and weevil resistance from first generation progeny tests in the Prince George breeding zone were selected for inclusion in an "add-on" clonal forestry strategy (RUSSELL & LOO-DINKINS 1993) using somatic emblings to enhance and deliver earlier genetic gains for interior spruce reforestation.

This study was undertaken to (i) assess the relative performance of emblings *versus* seedlings from a common genetic background, (ii) quantify genetic parameters associated with a population of clonal emblings, and (iii) estimate the genetic gain that might accrue from the use of emblings in a selection and deployment program under several testing and selection scenarios.

MATERIALS AND METHODS

Parental selection and breeding

Interior spruce first generation progeny trials were established in British Columbia (BC) in 1973 using open-pollinated progeny from 142 plus-tree parents selected in the low elevation (<1200 m) Prince George seed planning unit in central BC (KISS 1995; XIE & YANCHUK 2002). Forty-eight of the 142 parent trees were backward selected based on combined high weevil resistance and growth potential, and crossed at the Kalamalka Forestry Centre in Vernon, BC, in 1991 and 1992 to produce 50 full-sib families. Seed from these full-sib crosses were used to produce 1583 embling clones that were then established in seventeen clonal field trials in central BC between 1994 and 1998 (HAWKINS 1998).

This study examines two of the oldest of the 17 field trials containing 313 embling clones from 11 full-sib families. The number of clones per family ranged from 14 to 41. The 11 crosses were created from seven of the selected parents (Table 1).

Table 1. Crossing scheme, parent and full sib family identification numbers of the 11 crosses and 7 parents used at the Tumuch and Indian Point sites.

		Male parent					
		21	29	87	138	161	167
Female parent	1	1	2	5			10
	21		119			125	127
	87	65			107	73	75

Embling and seedling production

Embryos were excised from seed of the 11 full-sib crosses (families) and placed in an initiation medium for 4–12 weeks to produce embryogenic tissue which was multiplied by serial transfer to new petri plates over eight weeks. Tissues were then transferred to a maturation medium for up to six weeks, after which, mature embryos were harvested and stored. In early spring, 1995, mature embryos were placed on germination medium *in vitro* where they remained for six weeks. Germinants were then transferred into potting medium in 415B styroblocks and placed in a greenhouse in Maple Ridge, British Columbia, Canada, 50 km east of Vancouver (latitude 49°18'N). For more complete information regarding embling production, see GROSSNICKLE *et al.* (1996) or WEBSTER *et al.* (1990).

To compare the growth of emblings with that of seedlings, stratified seed from the same families as the emblings was sown in March 1995, concurrent with the transfer of embling germinants into styroblocks. The two stocktypes (emblings and seedlings) were grown in the same styroblock type and medium. Styroblocks of the two stocktypes were randomized together and re-randomized periodically throughout the growing season. Both stocktypes

were lifted in late fall of 1995 and cold-stored until planting the following spring.

Field tests and experimental design

Seedlings and emblings were planted in May 1996 at two field test sites (Indian Point and Tumuch) in central British Columbia. Indian Point (53° 29' N, 121° 35' W, 890 m elevation) is located 90 km southeast of Prince George in the Sub-Boreal Spruce biogeoclimatic subzone (MEIDINGER & POJAR 1991). Tumuch (53° 43' N, 121° 42' W, 990 m elevation) is located 70 km east of Prince George in the Interior Cedar Hemlock biogeoclimatic zone.

One seedling of each of the 11 families (except family 107, for which seedlings were unavailable) and one embling of each of the 313 clones were planted in a randomized complete block design in each of six (Indian Point) or eight (Tumuch) blocks. Of the 313 clones at the two test sites, 297 were represented at Indian Point and 294 at Tumuch.

Heights were measured each fall for seven years (except year six), commencing the year of sowing of seedlings and transfer of embling germinants into styroblocks (*i.e.*, HT1 to HT7).

Statistical analysis

i) Stocktype comparison

Growth differences between stocktypes were examined by testing the significance of stocktype on height at age-7 in analysis of variance using the following model in Proc Mixed (SAS 1997):

$$Y_{ijklm} = \mu + T_i + B_{j(i)} + F_k + S_l + C_{m(k)} + (TF)_{ik} + (TS)_{il} + (TC)_{im(k)} + (SF)_{lk} + (BF)_{kj(i)} + (BS)_{lj(i)} + (TSF)_{ilk} + (BSF)_{lkj(i)} + (BC)_{jm(ik)} \quad [1]$$

where Y_{ijklm} is the height of the m^{th} observation (either an embling clone or a seedling) of the l^{th} stocktype grown as the k^{th} family in the j^{th} block within the i^{th} test site. is the overall experimental mean. T_i, S_l, $B_{j(i)}$, F_k and $C_{m(k)}$ are the effects of test site, stocktype, block within site, family, and clone-within-family, respectively. All factors and interactions except T_i, S_l and $(TS)_{il}$ were considered random.

Isolating random effects involving clone (*i.e.* $C_{m(k)}$, $(TC)_{im(k)}$) posed a challenge since multiple clones exist in the embling condition of stocktype but not in the seedling condition. In addition, there was a severe degree of incompleteness (*e.g.*, between 14 and 41 clones per family per block; one seedling per family per block). These problems were addressed by first setting up dummy variables for the clones and test site-by-clone interaction and then adding separate random statements using the dummy variables to account for those two effects in the model. Dummy variables were assigned a common variance component by specifying the most basic Toeplitz structure (*e.g. type = Toep(1)* option) for both of the additional random statements.

Mean heights for the two stocktypes were determined using Best Linear Unbiased Estimates (BLUEs), and mean heights among clones and families were determined using Best Linear Unbiased Predictions (BLUPs). Both BLUEs and BLUPs were obtained using *estimate* statements in Proc Mixed.

Due to exponential growth of trees when young, the model above was also run with initial size (HT1, height when planted) of individual emblings and seedlings as a continuous covariate to account for size differences between stocktypes at planting, in order to assess growth independent of initial size.

ii) Genetic parameters and gain from mass clonal selection

Base or breeding populations are typically screened in progeny tests and superior parents or progeny identified and grafted into a clonal seed orchard in backward and forward selection schemes, respectively. However, an alternative or supplemental program of clonal testing and selection could provide additional genetic gains (RUSSELL & LIBBY 1986; FOSTER & SHAW 1988). The following section describes the methods we used to quantify additional gains that may be available from clonal testing, clonal mass selection and deployment of emblings.

The expected gain in age-7 height from selecting the tallest 20 clones regardless of family origin, based on 7-year heights, was estimated from hypothetical tests involving 200, 400, 800 and 2000 clones from 40 families, using genetic parameters estimated from the embling heights in the current study. The number of selected clones (20) from each hypothetical test was chosen to ensure that provincial guidelines for genetic diversity in seed and vegetative lots (*i.e.*, effective family number (N_e) of 10) would be satisfied. Since only emblings were considered in the analysis, *stocktype* and *family* effects and interactions involving these factors were omitted from the original model (Eq. 1). Variance components and other genetic parameters were derived for height at all measurement ages (1, 2, 3, 4, 5 and 7) using the restricted maximum likelihood (REML) estimator in Proc Mixed (SAS 1997).

To assess the magnitude of genetic variation among clones for height growth, the coefficient of clonal variation () was calculated as:

$$CV_c = 100 \times \frac{\sigma_c}{\bar{X}} \quad [2]$$

where is the square root of the estimated clonal component of variance and is the mean height (BLUE) of the 313 embling clones from the stocktype comparison. Repeatability of clonal means ($h^2_{\bar{C}}$) was calculated as:

$$h^2_{\bar{C}} = \frac{\sigma^2_C}{\sigma^2_{P\bar{C}}} \quad [3].$$

The phenotypic variance of clonal means ($\sigma^2_{P\bar{C}}$) was obtained using:

$$\sigma^2_{P\bar{C}} = \sigma^2_C + \frac{k_1\sigma^2_{TC}}{k_2} + \frac{\sigma^2_{BC}}{k_2} \quad [4]$$

where σ^2_C, σ^2_{TC} and σ^2_{BC} are the estimated variance components of clone, test site-by-clone, and block-by-clone, respectively. k_1 and k_2 are the expected mean squares coefficients for test site-by-clone (range 6.3 to 6.5) and clone (range 12.0 to 12.4) effects, respectively.

Expected gain using direct selection for height at age-7 (G_C) was estimated for the four testing scenarios:

$$G_C = ih^2_{\bar{C}}\sigma_{P\bar{C}} \quad [6]$$

where i, the selection intensity, ranged from 1.742 (20 of 200 clones selected) to 2.665 (20 of 2000 clones selected) (BECKER 1984).

Expected heights of the selected emblings were compared with the mean height (BLUE) of all tested clones to estimate the percent gain ($\%G_C$) that clonal testing, clonal mass selection and deployment of emblings would provide over conventional progeny testing, backward selection and seed deployment *via* a clonal seed orchard:

$$\%G_C = 100 \times \frac{(G_C - \bar{X})}{\bar{X}} \quad [7].$$

iii) Genetic parameters and gain from two-stage selection

Preliminary analyses of clonal height indicated that many of the tallest clones derived from a small number of families. To limit relatedness among selections, genetic gain was also estimated for two-stage selection (family plus clone-within-family selection) (COTTERILL 1986) - first selecting the tallest 20 of 40 families ($i = 0.782$), and then selecting the tallest clone within each of the 20 selected families. The analysis was performed for hypothetical tests containing 200, 400, 800 and 2000 clones, that is, for tests containing 5, 10, 20 and 50 clones per family, respectively (*i.e.*, $i = 1.163$, 1.539, 1.876 and 2.249, respectively).

Variance components were estimated for two-stage selection by applying the full model (Eq. 1) to the embling data with effects for *stocktype* and its interactions removed. The magnitude of genetic variation among *families* (CV_F) and among *clones-within-families* ($CV_{C(F)}$) was calculated in the same fashion as CV_C (Eq. 2), replacing σ_C with σ_F and $\sigma_{C(F)}$, respectively.

Phenotypic variation of *family* means ($\sigma^2_{P\bar{F}}$) was calculated using the expected mean squares for *family* (MULLIN *et al.* 1992), obtained from Proc GLM

$$\sigma^2_{P\bar{F}} = \sigma^2_F + \frac{k_3\sigma^2_{TF}}{k_5} + \frac{k_4\sigma^2_{BF}}{k_5} + \frac{\sigma^2_{BC}}{k_5} \quad [8]$$

where k_3, k_4 and k_5 are the expected mean squares coefficients of *test site-by-family* (range 162.2 to 169.5), *block-by-family-within-test site* (range 23.9 to 24.6), and *family* (range 324.2 to 338.8), respectively.

Phenotypic variation of *clone-within-family* means ($\sigma^2_{P_{C(F)}}$) was calculated using the expected mean squares for *clone-within-family*:

$$\sigma^2_{P_{C(F)}} = \sigma^2_C + \frac{k_6\sigma^2_{TC}}{k_7} + \frac{\sigma^2_{BC}}{k_7} \quad [9]$$

where k_6 and k_7 are the expected mean squares coefficients of *test site-by-clone-within-family* (range 6.3 to 6.5) and *block-by-family-within-test site* (11.9 to 12.3), respectively. *Family* (h^2_F) and *clone-within-family* ($h^2_{C(F)}$) repeatabilities were calculated by dividing the *family* and *clone-within-family* variance components by their respective phenotypic vari-

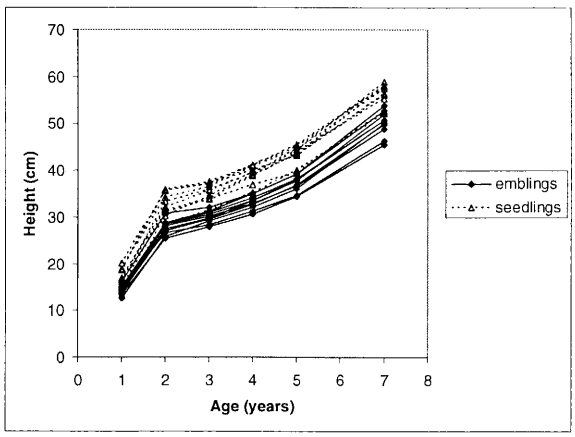

Fig. 1. Height – age curves for interior spruce families grown as both emblings and grown at two sites in central British Columbia.seedlings. Values are best linear unbiased predictions for materials.

Table 2. Height of interior spruce emblings and seedlings at age 7 (HT7) with and without first year height (HT1) used as a covariate. Values are best linear unbiased estimates at two test sites in central British Columbia. Also shown is the significance of stocktype and HT1 effects.

HT 7 (cm)		p > F		
Embling	Seedling	Stocktype	HT1	
50.1	54.5	0.0414	0.0001	covariate
49.9	56.2	0.0105	–	no covariate

ances.

Genetic gains using direct selection of height at age-7 were predicted separately for *family* (G_F) and *clone-within-family* ($G_{C(F)}$) using Eq. 6 (substituting the corresponding heritabilities and phenotypic standard deviations). The two gain values were added to obtain the predicted total genetic gain (G_T). Percent gain for two-stage selection ($\%G_T$) was calculated as for clonal mass selection (Eq. 7), but replacing G_C with G_T.

RESULTS

Stocktype comparison

Height growth was slow at both test sites. After seven seasons, emblings (49.9 cm) were significantly shorter than seedlings (56.2 cm) (difference = 11 %; p = 0.0105), however, the difference between stocktypes was reduced to 8 % (p = 0.0414) when first year height (*HT1*) was accounted for (Fig. 1 and Table 2). In spite of differences in initial height, growth trajectories for emblings and seedlings appear to be parallel after the first two years (Fig 1).

Pronounced planting shock during the third growing season (second field season) is evident in the height-growth curves (Fig. 1). Nonetheless, survival at age-7 was excellent for both emblings (97 %) and seedlings (99 %). There were no signs of attack by white pine weevil (*Pissodes strobi* (Peck)) during the seventh growing season at Indian Point and signs were infrequent (6 % of emblings; 10 % of seedlings) at Tumuch.

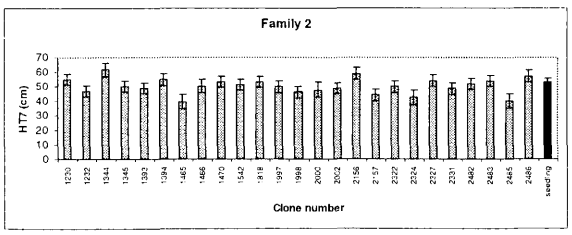

Fig. 2. Best linear unbiased predictions of age–7 height and standard errors of embling clones and seedlings of one of the 11 full-sib families of interior spruce at two test sites in central British Columbia.

Genetic parameters

Large and significant clonal variation within fami lies was present (Fig. 2, heights of only one family are shown; other families displayed similar levels of within-family variability), and is quantified in the large clonal mean coefficients of variation (*e.g.*, CV_C = 14.6 at age 7, Table. 3). Repeatabilities (*e.g.*, 0.71 at age 7) were also strong, but both variation and repeatabilities generally declined with age (Table 3).

Genetic variation among *families* (CV_F = 4.5–6.3 %) was less than half that of *clones-within-families* ($CV_{C(F)}$ = 12.0–20.2 %) (Table 4). Repeatabilities, however, were equally strong for *families* (h^2_C = 0.80–0.97) and *clones-within-families* ($h^2_{C(F)}$ = 0.69–0.93). Values of all genetic parameters generally declined with age.

Estimated genetic gain

The estimated percent genetic gain ($\%G_C$) for direct selection for age-7 height reflects the large genetic variation and strong heritability: 21.5 % in tests in which 20 of 200 clones were selected, increasing to 32.8 % in tests in which 20 of 2000 clones were selected (Fig. 3). Estimated total percent genetic gain ($\%G_T$) for direct selection for height at age-7 using two-stage selection increased with test size (17.0 % with 200 clones tested; 29.0 % with 2000 clones tested), but was consistently about 4 % smaller at each test size than when mass clonal selection was used (Fig. 3).

DISCUSSION

Stocktype comparison

While stocktype heights differed significantly, the differences were relatively small. The significant effect of the covariate (*HT1*; $p < 0.0001$) indicates that HT7 is affected by HT1, and a substantial part of the height difference between the two stocktypes at age-7 is related to stocktype height at planting. Differences in growth rate do not appear to be increasing after the second growing season (Fig. 1).

These results are corroborated by another study of interior spruce, in which height and diameter were

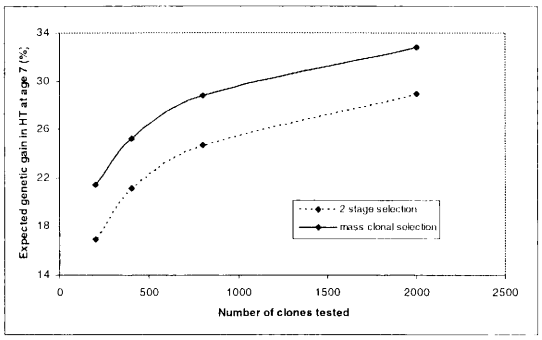

Fig. 3. Expected percent genetic gain for direct selection for age–7 height arising from the selection of 20 clones and their deployment as emblings, based on testing schemes involving 200 to 2000 test clones using clonal mass selection and two-stage selectiona for interior spruce in central British Columbia.

also smaller in emblings than in seedlings at the end of the first and second growing seasons (GROSSNICKLE & MAJOR 1994). Furthermore, height increment during the second season in their study was the same for the two stocktypes, suggesting that emblings and seedlings have similar growth potentials.

Continued research into developing laboratory and nursery techniques for embling production could potentially result in improved early growth (FRAMPTON & FOSTER 1993). Refinement of laboratory (MERKLE *et al.* 1997; ADERKAS & BONGA 2000; PERCY *et al.* 2000; HÖGBERG *et al.* 2001; PULLMAN & JOHNSON 2002; WEYERHAEUSER 2003) and nursery protocols (GROSSNICKLE *et al.* 1996; SUMMERS & CALAM 1998; SUTTON 2002) since 1993 when the emblings used in the present study were cultured, suggests that it may currently be possible to produce emblings and seedlings of comparable initial size for reforestation.

Heavy vegetation competition and several severe frost events at Tumuch, and compacted soils at Indian Point may have contributed to the poor growth of the trees in this experiment. Nonetheless, genetic variation was discernable, as evidenced by the strong repeatabilities. Height growth of interior spruce is typically greater on most reforestation sites in this region (VYSE 1981; KISS & YANCHUK 1991) than in the present study.

Genetic parameters and gain predictions

Results of this study illustrate that significant genetic gains in height at age-7 may be achievable through clonal testing, selection and deployment of interior spruce emblings. Sizeable genetic gains using clonal selection and deployment have also been predicted for other conifer species (PARK & FOWLER 1987; SHELBOURNE 1992; MULLIN & PARK 1994) due to the ability of clonal programs to capture additional additive and non-additive genetic variation (LIBBY & RAUTER 1984). Furthermore, clonal deployment bypasses the seed orchard development stage, thus potentially increasing gain per unit time (MATHESON & LINDGREN 1985; SHELBOURNE 1992).

The emblings used in this study were derived from crosses among a set of elite parents. Use of the same parents in a seed orchard would would therefore yield a seedlot genetically equivalent to the embling population tested in this study. Consequently, estimated gains from selection within the embling population are gains that would be expected beyond that of a conventional seed orchard program. However, this rationale assumes emblings and seedlings of similar genetic constitution will display equivalent growth. In the present study, emblings were significantly shorter than seedlings. Therefore, the estimated gains are contingent on improvements to laboratory and nursery procedures that will result in stocktypes of equivalent size, as discussed in the *stocktype comparison* section above. If stocktype differences remain unchanged from 1993 when the emblings were cultured, clonal testing and selection would result in gains of approximately half the magnitude of those estimated in this study.

The sizeable genetic gains estimated in the present study are mainly a consequence of both large clonal variation and strong clonal and family repeatabilities. Reports of clonal variation for height were smaller for rooted cuttings of Sitka spruce (*Picea sitchensis* (Bong.) Carr.)(CV_C = 7.7 %) at age 11 (Costa e Silva *et al.* 1994), but comparable to that of rooted cuttings of Norway spruce (*Picea abies* (L.) Karst.) (CV_C = 12.3 %) at age 7 (LEPISTO 1993). In a study with interior spruce clones derived from rooted cuttings, similar levels of variation were found as in the current study (CV_C = 11.9 % at age 6; J. Russell unpubl.data).

Genetic variance and heritabilities were strongest in the first two years of the study. Other reports also show a sharp decline in genetic variance and heritabilities after the seedling stage in interior spruce (XIE & YANCHUK 2002). While gains for direct selection on young seedlings (2–3 years old) may therefore exceed gains for direct selection at age-7, gains at rotation are typically less for indirect selection on young seedlings because genetic correlations between growth of young seedlings and rotation age growth are expected to be weak (LAMBETH 1980).

Table 3. Variance components, phenotypic variation of clonal means (σ^2_{PC}), coefficient of variation among clones (*CVc*), clonal repeatability (h^2_C), and best linear unbiased estimate of height at ages 1, 2, 3, 4, 5 and 7 for 313 interior spruce embling clones growing at two test sites in central British Columbia.

	Selection age					
	1	2	3	4	5	7
	Height of tested emblings at selection age (cm)					
	14.4	27.6	29.9	33.0	37.0	49.9
σ^2_B	0.36	0.13	0.35	0.46	1.18	7.48
σ^2_C	9.26	20.63	17.28	18.95	22.77	53.19
σ^2_{TC}	0.17	1.00	1.47	2.12	2.05	11.52
σ^2_{BC}	7.22	12.39	20.07	28.52	48.43	185.84
CV_C	21.2	16.4	13.9	13.2	14.5	14.6
σ^2_{PC}	9.9	22.2	19.7	22.4	27.8	74.8
h^2_C	0.93	0.93	0.88	0.85	0.82	0.71

Strong clonal mean repeatabilities in this study corroborate estimates in other species (PARK & FOWLER 1987; LEPISTO 1993; LAMBETH *et al.* 1994; MULLIN & PARK 1994). While repeatabilities are similar for *clones-within-families* and for *families* in the present study, as well as in a study involving black spruce (*Picea mariana* (Mill.) B.S.P.) (MULLIN *et al.* 1992), variation is substantially larger for *clones-within-families*. Consequently, gains will be greatest when testing and selection can focus on clones rather than on families.

Clone-by-site variance relative to that of the total variance, was small (3–4 %), and similar to that of other studies (SHAW *et al.* 1988; LEPISTO 1993; LIN & ZSUFFA 1993). Minimal *clone-by-site* variance is supported by a strong genetic correlation between the two sites (r_b = 0.78) (BURDON 1977). Levels of genotype-by-environment variation of this magnitude indicate that clonal ranking should be stable when emblings are grown on sites comparable to those used in the present study.

Substantial relatedness exists among the tallest 20 clones: 14 (70 %) belong to only two families (Table 5), and several of the families, including the two with the tallest clones, have a common parent (Table 1). Consequently, if selections were to be made from among the 313 clones considered in this study, more clones would need to be selected, more families tested, or some form of *family* and *clone-within-family* selection will be required to reduce relatedness among selections and satisfy provincial guidelines for minimum effective family size (N_e) of 10. Furthermore, differences among clones in bulking-up rates and outplanting survival increase the number of clones needed to be included in a production program. Experience with a clonal testing program in yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach) indicates that 30 selected clones results in an N_e of 20, given relatedness of clones and uneven bulking-up (J. Russell unpubl.data). Increased genetic gain can also be realized through the unequal deployment of more clones without decreasing levels of diversity (LINDGREN *et al.* 1989).

Challenges facing somatic embryogenesis

While the potential genetic gain for height from the selection and deployment of emblings appears promising, several technical challenges remain to be resolved prior to widespread incorporation of SE technology into reforestation programs. First, somaclonal variation (mutations arising in somatic cells that are propagated as the organism grows) in cyropreserved embryos can result in failure to achieve expected gains. Gene mutations (DEVERNO *et al.* 1999) and polyploidy (FOURRE *et al.* 1997) have been cited as the possible causes of somaclonal variation. Second, recalcitrance to SE procedures among some genotypes may restrict which clones or families can be propagated as emblings (BECWAR 1990; PARK *et al.* 1993).

Third, indirect selection for unfavourable growth or adaptive traits could occur during somatic

Table 4. Variance components, phenotypic variation of families (s_{PF}^2) and clones within families ($s_{PC(F)}^2$), coefficient of variation among families (CV_F) and among clones within families ($CVC_{(F)}$), family heritability (h^2_F) and clonal repeatability ($h^2_{C(F)}$), and best linear unbiased estimate of height at ages 1, 2, 3, 4, 5 and 7 for 313 interior spruce embling clones growing at two test sites in central British Columbia.

	Selection age					
	1	2	3	4	5	7
	Height of tested emblings at selection age (cm)					
	14.4	27.6	29.9	33.0	37.0	49.9
σ^2_B	0.36	0.13	0.34	0.45	1.18	7.46
σ^2_F	0.83	2.59	1.82	2.22	2.96	8.71
σ^2_{TF}	0.00	0.15	0.31	0.44	0.32	3.23
σ^2_{BF}	0.00	0.00	0.11	0.13	0.03	0.45
$\sigma^2_{C(F)}$	8.46	17.95	15.35	15.35	19.78	44.09
$\sigma^2_{TC(F)}$	0.17	0.87	1.17	1.17	1.74	8.25
σ^2_{BC}	7.22	12.40	19.98	19.98	48.43	185.50
CV_F	6.3	5.8	4.5	4.5	4.6	5.9
σ^2_{PF}	0.85	2.70	2.04	2.53	3.26	10.93
h^2_F	0.97	0.96	0.89	0.88	0.91	0.80
$CV_{C(F)}$	20.2	15.3	13.1	12.3	12.0	13.3
$\sigma^2_{PC(F)}$	9.14	19.42	17.63	19.85	24.70	64.05
$h^2_{C(F)}$	0.93	0.92	0.87	0.84	0.80	0.69

Note> See Equation 1 for description of variance components.

embryo-genesis-cryopreservation if the various steps of the procedure result in a selective loss of families or clones. A correlation between embryogenic and growth/adaptive traits was not observed in Norway spruce where recovery of families and clones-within-families was relatively good (HÖGBERG *et al.* 1998), however, poor recovery in many facilities will require continued attention to avoid indirect selection. Fourth, the success of an SE program also depends on the ability of selected clones to be bulked-up in sufficient quantity (GROSSNICKLE *et al.* 1996) and without complication after 8-12 years of cryopreservation while clonal field tests are conducted. To our knowledge, no information exists regarding this issue.

However, the major obstacle facing SE programs may be economics. The cost of developing a somatic embryogenesis system, additional testing, cryopreservation, bulking-up and nursery production of elite clones may be prohibitive. While opportunities for reducing costs may exist though economies of scale in a large operational program, current costs of emblings have remained approxi mately double that of seedlings in BC for the last decade. In Ontario, an economic analysis of seed orchard *versus* clonal tree improvement with black spruce concluded that clonal forestry should not be a major part of reforestation unless volume gains (clonal over seed orchard) at rotation exceed 20–50 % or stocktype costs are equivalent (MCKENNEY *et al.* 1989). Prospects for clonal forestry, however, may be better in regions where growth rates are faster, and returns on investment arrive earlier.

Our study suggests that there may be a significant opportunity to capture growth gains in excess of that already available from seed orchards through testing, selection and deployment of interior spruce embling clones. Substantial genetic variation in weevil resistance has been detected in interior spruce (KING *et al.* 1997). As current embling field trials age and weevil attacks become more

Table 5. Height (cm) at age 7 (HT7) of the tallest 20 interior spruce clones from among the 313 tested clones estimated as best linear unbiased predictions. Note that 14 (70 %) of the clones are from just two families (65 and 75) and that these two families share a common parent (see Fig. 1).

Rank	Family	Clone	HT7
1	65	2165	62.7
2	65	1989	62.0
3	75	1750	61.9
4	2	1344	61.8
5	75	1271	61.5
6	75	1360	61.2
7	65	2094	61.0
8	73	2354	60.6
9	65	2166	59.9
10	65	1960	59.6
11	125	2254	59.5
12	75	1654	59.4
13	75	1743	59.0
14	2	2156	58.9
15	65	1970	58.7
16	65	1981	58.5
17	125	2253	58.4
18	73	1724	58.0
19	65	2080	57.9
20	65	1845	57.8

pronounced, additional genetic gain is also likely to be available from clonal selection and deployment of weevil resistant emblings. An economic evaluation is needed to determine the additional stock cost that would be justified in order to access the potential gains from SE forestry in BC.

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