Genetic Parameters of Somatic Clones of Coastal Douglas-fir for Growth, Stem and Wood Traits at $6^1/_2$ or $7^1/_2$-Years in Washington and Oregon, USA

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Abstract

Five genetic tests involving 70 somatic clones of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) were planted March–April 1999 in Weyerhaeuser plantations across western Washington and Oregon states, USA. Four of the tests are in the Longview and Twin Harbors regions of Washington, and one test is in Springfield, Oregon. Each test is designed as single-tree plots with 12 randomized complete-blocks. The 70 coastal Douglas-fir clones were propagated by somatic embryogenesis (SE) from two full-sib families created by crossings among three parents. Results are reported for height, diameter at breast-height (DBH) and stem volume measured at $7^1/_2$-years; and stem sinuosity, stress wave velocity (SWV) and pilodyn at $6^1/_2$-years. Within-family clonal heritabilities (or repeatabilities) were estimated as the ratio of the variance between-clones within-families to the overall phenotypic variance. Variance between families was not included in the numerator of the heritability equation because the 70 SE clones are from only two full-sib families.

Height had a within-family clonal heritability of 0.31 ± 0.04, DBH 0.27 ± 0.04, volume 0.24 ± 0.04, stem sinuosity 0.13 ± 0.02, SWV 0.45 ± 0.04 and pilodyn 0.31 ± 0.04. The three growth traits were all closely genetically associated with clonal correlations among them of 0.86 to 0.98. Clonal performance for growth proved quite stable across tests with an overall between-test clonal correlation of 0.80 ± 0.04 for stem volume, meaning that clone x test interactions only accounted for a minor part of the total variance. The between-test correlation was 0.79 ± 0.06 for sinuosity, 0.96 ± 0.01 SWV and 0.86 ± 0.03 for pilodyn.

Key words: Coastal Douglas-fir, *Pseudotsuga menziesii*, somatic embryogenesis, adaptability, within-family clonal heritability, clonal stability, wood properties.

Introduction

Coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) is an important commercial forest species in North America and has been a major focus of genetic improvement. Weyerhaeuser manages one of the most advanced genetic improvement programs of coastal Douglas-fir that began in the mid-1950s to improve growth, stem and wood quality (STONECYPHER *et al.,* 1996; DEAN and STONECYPHER, 2006; DEAN, 2007; DEAN, 2008; DEAN *et al.,* 2009). Weyerhaeuser's coastal Douglas-fir breeding activities are today well into the third generation of selection and testing. A core part of Weyerhaeuser's advanced-generation improvement strategy is the use of somatic embryogenesis (SE) and manufactured seed technologies to bring more concentrated investment on elite genotypes in nucleus breeding populations (DEAN, 2007; DEAN, 2008; DEAN *et al.,* 2009).

SE is an *in vitro* tissue culture technique that involves repeating the normal conifer polyembryony process in zygotic seed embryo development to produce genetically identical embryos. These embryos can be grown *ex vitro* as seedlings in a nursery to produce clonal trees for genetic testing and plantation establishment. NAGMANI *et al.* (1991) and GUPTA *et al.* (1994) reported various aspects of SE technologies applied specifically to cloning Douglas-fir. The integration of SE technologies into genetic improvement programs raises possibilities of enhanced genetic gain through more rapid and flexible deployment of superior genotypes, as well as long-term storage of embryo tissue in cryopreservation (CHELIAK and ROGERS, 1990; PARK *et al.,* 1998; BENOWICZ *et al.,* 2002; HARGREAVES *et al.,* 2002; SUTTON, 2002; ALLAN, 2003; HÖGBERG, 2003; CYR *et al.,* 1994; GROSSNICKLE and FOLK, 2005).

Thorough analysis of genetic improvement strategies with SE and the integration of SE technologies into conventional genetic improvement programs require reliable estimates of genetic parameters including heritabilities, genetic variances and correlations. These genetic parameters are required to: (i) estimate gains from SE clonal selection on specific traits and indirect correlated changes in other traits, (ii) optimise genetic testing, and (iii) design appropriate selection and breeding strategies. DEAN (2008) and DEAN *et al.* (2009) present estimates of within-family clonal genetic parameters for growth and stem sinuosity at $5^1/_2$ to $7^1/_2$-years after planting across a range of genetic tests of somatic clones of coastal Douglas-fir in western Washington and Oregon States, Pacific Northwest USA.

The study reported here is based on five field tests established in 1999 across Washington and Oregon, and involving 70 SE clones of coastal Douglas-fir propagated from two full-sib families. DEAN (2008) has reported genetic parameters for growth traits only at $5^1/_2$-years for the same 1999 series of genetic tests. The current study presents clonal genetic parameters estimated on a within-family basis for stem and wood quality traits at $6^1/_2$-years after planting, as well as growth at $7^1/_2$-years. These appear to be the first published estimates of genetic parameters of SE clones for wood quality of Douglas-fir and are within-family clonal genetic parameters that do not include variance between-families.

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Experimental Details

Sites, Establishment and Silviculture

The five genetic field tests were planted March–April 1999 in Weyerhaeuser Company plantations across western Washington and Oregon states, and included 70 clones of coastal Douglas-fir propagated from two full-sib families using SE (GUPTA *et al.,* 1994). Tests identified as LV1 and LV2 are in the Longview region of Washington; TH3 and TH4 in Twin Harbors, Washington; and SP5 in Springfield, Oregon. The tests were planted by hand using containerized seedlings at 3,020 trees per hectare (1.82 m x 1.82 m). Site and establishment details of all five genetic tests are summarised in *Table 1*.

Genetic Material

The 70 coastal Douglas-fir clones were propagated by SE from two full-sib families that were related through a common female parent: 43 clones were propagated from family AxB (female A x male B); and 27 clones from family AxC. All three parents A, B and C were first-generation plus trees selected from natural stands of coastal Douglas-fir in Longview (DEAN, 2008).

Somatic Embryogenesis Technology

The somatic seedlings were produced using SE procedures and media for Douglas-fir as described by GUPTA *et al.* (1994). In summary, the protocol started with excising immature zygotic embryos from variable numbers of seeds of each full-sib family. Embryonic suspensor masses (ESM) were then initiated *in vitro* from the individual excised embryos using a semi-solid medium containing mineral nutrients, sucrose and vitamins. The next step involved multiplication of the ESM cultures by weekly subculture in fresh liquid medium. An *in vitro* development step followed in which mature somatic embryos were produced on pads soaked in high osmolality liquid development medium containing abscisic and gibberellic acid, as well as activated charcoal (GUPTA *et al.,* 1994).

The germinants were then transferred to *ex vitro* nursery conditions. This transfer involved the individual selection of good quality (based on size and morphology) somatic germinants with epicotyls. These germinants were transplanted into a mixture of peat, vermiculite and perlite in 164 cm^3 "supercell" containers. All somatic seedlings were grown in the same greenhouse for one year and had morphology and growth rates within the normal range exhibited by zygotic seedlings.

Field Design

All five genetic tests were designed as single-tree plots of the 70 somatic clones randomised across 12 complete-replicates.

Measurements

In December 2006 (at over $7\frac{1}{2}$-years after planting) all surviving trees in all tests were measured for height using poles; and stem diameter over-bark at breast-height (DBH at 137 cm) using diameter tapes. Stem volume over-bark was estimated from height and DBH for each tree using the small-tree volume equation developed by BRUCE and DEMARS (1974). Survival was calculated as the percent of trees planted that survived to $7\frac{1}{2}$-years.

Stem sinuosity was assessed in December 2005 (at about $6\frac{1}{2}$-years) by estimating deflection of the stem from vertical using the following five scores: Score 0 = straight, score 0.25 = 0.25 inch (0.64 cm) deflection, score 0.5 = 0.50 inch, score 1 = one inch, and score 2 = two inches (5.08 cm) or more.

Stress wave velocity (SWV) and pilodyn were measured on all standing trees in October 2005 ($6\frac{1}{2}$-years) across the five tests. SWV was measured over a one-metre section of the tree's stem (from 0.5 to 1.5 metres above ground level) using a TreeSonic timer. The TreeSonic timer is a patented acoustic device designed to measure stress wave propagation in standing trees (HUANG, 2005). A signal is generated by a hammer tap on one sensor and the time it takes to reach a second sensor is recorded. The distance between the two sensors (in this case one metre) is used to calculate the velocity in the fibre direction. Fibre direction velocity correlates well with Modulus of Elasticity (MOE) of wood, with greater SWV indicating greater MOE (ANONYMOUS, 2006).

Table 1. – Site parameters for genetic tests LV1 and LV2 (Longview, Washington); TH3 and TH4 (Twin Harbors, Washington); and SP5 (Springfield, Oregon).

	LV1	LV2	TH3	TH4	SP5
Plantation	Hemlock Creek	Ostrander Creek	Dryad-Dell Creek	Goat Mtn – J Line Creek	Anderson Creek
County, State	Cowlitz Co., Washington	Cowlitz Co., Washington	Lewis Co., Washington	Pacific Co., Washington	Lane Co., Oregon
Elevation (m)	320	460	180	180	370
Slope	10%	level	10%	15%	30-40%
Soil Association	Morgan	Morgan	Newlund	Astoria	Nekia
Soil texture	Clay loam	Clay loam	Silty clay	Silty loam	Clay
Site index	130	130	140	140	120

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Two pilodyn pin penetration measurements were also made on each tree; the first was recorded just below breast-height and the other just above breast-height. The pilodyn measurement for each tree in this study was taken as the average of the two recordings. Pilodyn has an inverse association with wood specific gravity (SG) with greater pilodyn pin penetration indicating lower wood SG.

Statistical Methods

(1) Statistical Package: All analyses were carried out using the ASREML (GILMOUR *et al.,* 2006) statistical package which is based on "Restricted Maximum Likelihood" methodology (PATTERSON and THOMPSON, 1971; SEARLE *et al.,* 1992). ASREML makes optimal use of all available information, has considerable flexibility in the range of models that can be fitted and its solutions for random effects are Best Linear Unbiased Prediction (BLUP).

(2) MODEL 1: Was fitted to growth, stem and wood data for the 70 somatic clones (from two families) and used to estimate within-family clonal variance components for each trait – (1)

$$\text{TRAIT}_{ijklm} = \mu + \text{TEST}_i + \text{REP}_{ij} + \text{FAMILY}_k + \text{CLONE}_{kl} + \text{CLONE.TEST}_{ikl} + \text{RESIDUAL}_{ijklm} \quad (1)$$

where TRAIT_{ijklm} is the observation on the m^{th} tree of the l^{th} SE clone from the k^{th} full-sib family and growing in the j^{th} replicate of the i^{th} test; μ is a fitted mean; TEST_i is the effect of the i^{th} genetic test (i = 1–5), assumed to be a fixed effect; REP_{ij} is the random effect of the j^{th} replication nested within the i^{th} test (j = 1–12); FAMILY_k is the fixed effect of the k^{th} full-sib family (k = 1–2); CLONE_{kl} is the random effect associated with the l^{th} SE clone nested within the k^{th} full-sib family (l = 1–43 in family AxB and 44–70 in family AxC); CLONE.TEST_{ikl} is the interaction of clone and test; and RESIDUAL_{ijklm} is a residual error among the m^{th} trees within the l^{th} clone. The effect of FAMILY_k is considered fixed in MODEL 1 because two families are not sufficient to reliably estimate a variance component for this effect. Preliminary analyses showed that the interaction of family x test was not statistically significant for any of the traits studied and so this interaction term was not included in MODEL 1.

It is important to note that any propagation effects that may be associated with particular clones but not of genetic origin ("c-effects") are completely confounded with CLONE_{kl}. C-effects are commonly attributed to environmental factors associated with the rooting of the clone (ISIK *et al.,* 2003; BALTUNIS *et al.,* 2005). There appear to be no published studies examining c-effects in somatic Douglas-fir trees.

MODEL 1 was used to estimate variance components for the effects of CLONE nested within-families (σ^2_C within-families), CLONE.TEST interaction (σ^2_{CT}) and the RESIDUAL within-clone error (σ^2_e). Overall phenotypic variance (σ^2_P) was estimated as – (2)

$$\sigma^2_P = \sigma^2_C + \sigma^2_{CT} + \sigma^2_e \quad (2)$$

Within-family clonal heritabilities (H^2_C) or within-family clonal repeatabilities were defined as the ratio of between-clone (within family) variance to phenotypic variance – (3)

$$H^2_C = \sigma^2_C / \sigma^2_P \quad (3)$$

Between-family variance has deliberately been taken out of the numerator of *Equation 3* because two full-sib families of common female parentage is not a sufficient sample to estimate variance for the effect of family. The H^2_C estimated by *Equation 3* is actually a repeatability parameter that gives an indication of the degree to which a somatic clone's superiority is repeatable, relative to other clones from the same family. The terms "clonal heritability" and "clonal repeatability" are both commonly used to describe H^2_C.

In preliminary analyses H^2_C was estimated with between-family variance included in the clonal variance (σ^2_C) component. In this case σ^2_C represents variance among SE clones ignoring the fixed effects of families, and H^2_C the repeatability of a clone's superiority ignoring family. These estimates of H^2_C ignoring family were almost identical to corresponding within-family parameters. This observation reflects the similarity of the two particular full-sib families involved in this study with respect to average performance of their clones. It is important to note that the current study does not include sufficient families to reach reliable conclusions about the influence of family on performance of somatic Douglas-fir clones.

(3) MODEL 2: This model is a variant of MODEL 1 and was used to simultaneously estimate parameters specific to each individual test. In MODEL 2 the CLONE (σ^2_C) and CLONE.TEST (σ^2_{CT}) variances were partitioned into separate but uniformly correlated components for each test. Expressed another way, it was assumed that the correlation structure between all pairs of tests was uniform. Separate residual variances were also fitted to each test to allow site-specific estimates of within-family clonal heritability and between-test clonal correlations. A between-test correlation of unity implies that the SE clones rank the same across each of the five tests. A correlation of zero implies that there is no correspondence between the rankings of clones across the tests.

(4) MODEL 3: Is another variant of MODEL 1 and was used to estimate between-trait clonal correlations on a pair-wise basis. This was done by adding an extra dimension to each term to accommodate bivariate structures, and also excluding the interaction terms. Two variances and a covariance were fitted for the replicate (within-test), clone (within-family) and error terms. Thus MODEL 3 accommodates a two trait vector/matrix of effects across all tests, while MODEL 1 is univariate across tests.

Results and Discussion

General

Total numbers of SE trees planted, their average survival, height and wood properties across the five genetic tests LV1, LV2, TH3, TH4 and SP5 are given in *Table 2.* Survival to 7½-years was highest at Twin Harbors (TH3, TH4) and Springfield (SP5) with test mean sur-

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Table 2. – Numbers of somatic trees planted, and their mean survival and stem height at $7\frac{1}{2}$-years; and stress wave velocity (SWV) and pilodyn at $6\frac{1}{2}$-years across genetic tests LV1, LV2, TH3, TH4 and SP5.

	LV1	LV2	TH3	TH4	SP5
Clones planted (no.)	70	70	70	70	69
Trees planted (no.)	840	840	840	840	828
Trees measured (no.)	769	778	816	834	813
Mean survival (%)	92%	93%	97%	99%	98%
Mean tree height (m)	5.2	4.6	5.4	6.1	5.7
Mean SWV (m/s)	2583	2438	2395	2465	2317
Mean pilodyn (mm)	11.2	11.6	13.2	13.8	13.3

vivals ranging from 97% to 99% (*Table 2*). The Longview tests had the lowest survival of 92% and 93% in LV1 and LV2, respectively. Test LV2 grew more slowly with mean height 4.6 m at $7\frac{1}{2}$-years for SE trees, compared with 5.2 to 6.1 m across the other tests (*Table 2*). Pilodyn was higher (indicating lower wood specific gravity, SG) in the Twin Harbors (TH3, TH4) and Springfield tests SP5 (*Table 2*).

Clonal Variances and Heritabilities

(1) Growth: Table 3 presents ASREML estimates of variance components and heritabilities using fitted MODEL 1 for growth, stem sinuosity, stress wave velocity (SWV) and pilodyn at $6\frac{1}{2}$ or $7\frac{1}{2}$-years of the 70 SE clones across the five Washington and Oregon tests. *Table 4* presents ASREML estimates with fitted MODEL 2 of within-family clonal heritabilities for each individual genetic test. Of the growth traits, height had the highest within-family clonal heritability of $H^2_C = 0.31 \pm 0.04$ (*Table 3*), with values ranging from $H^2_C = 0.37$ to 0.42 ± 0.05 in individual tests (*Table 4*). The overall estimate of H^2_C across all tests is lower than site-specific estimates because of imperfect between-test correlations due to clone x test interactions (discussed later). Within-family clonal heritabilities for diameter at breast-height (DBH) at $7\frac{1}{2}$-years were lower than height with $H^2_C = 0.27 \pm 0.04$ (*Table 3*); values ranging from 0.31 to 0.37 ± 0.05 in individual tests (*Table 4*). The heritability of volume followed DBH with $H^2_C = 0.24 \pm 0.04$ (*Table 3*). It is interesting to note that the level of between-clone variance (σ^2_C) is at least three times as large as the clone x test interaction (σ^2_{CT}, *Table 3*); indicating little interaction among SE clones for growth to $7\frac{1}{2}$-years (discussed later).

These estimates of H^2_C for growth traits imply that appreciable SE clonal genetic variation exists within families for growth of coastal Douglas-fir, and this variation is available for capture in SE clonal programs. The H^2_C presented in this paper give an indication of the degree to which a somatic clone's superiority across tests is repeatable, relative to other clones from the same family. As already mentioned, any c-effects that may be present in this study are completely confounded with H^2_C, and these effects may lead to substantial bias in the genetic parameter estimates.

DEAN (2008) reported somewhat lower levels of within-family clonal heritability for height ($H^2_C = 0.25 \pm 0.01$),

Table 3. – ASREML estimates and standard errors (se) determined with fitted MODEL 1 for overall means, variance components and within-family clonal heritabilities for stem height, diameter at breast-height (DBH) and stem volume at $7\frac{1}{2}$-years; and stem sinuosity, stress wave velocity (SWV) and pilodyn at $6\frac{1}{2}$-years for coastal Douglas-fir somatic clones across the genetic tests LV1, LV2, TH3, TH4 and SP5.

	Height (m)	DBH (mm)	Volume (dm^3)	Sinuosity (score)	SWV (m/s)	Pilodyn (mm)
Overall Means:						
Mean ($\mu \pm$ se)	5.44 ± 0.06	64.0 ± 1.0	9.64 ± 0.30	0.14 ± 0.01	2438 ± 17	12.6 ± 0.2
Variance Components:						
Clones within-families ($\sigma^2_C \pm$ se)	0.21 ± 0.04	51.4 ± 9.7	4.49 ± 0.86	0.005 ± 0.001	16330 ± 2902	0.85 ± 0.16
Clone x test interaction ($\sigma^2_{CT} \pm$ se)	0.05 ± 0.01	14.6 ± 2.2	1.37 ± 0.21	0.003 ± 0.001	1896 ± 423	0.61 ± 0.12
Within-clone residual ($\sigma^2_e \pm$ se)	0.40 ± 0.01	124.8 ± 3.0	12.7 ± 0.30	0.029 ± 0.001	961 ± 242	0.17 ± 0.03
Phenotypic variance ($\sigma^2_P \pm$ se) [A]	0.66 ± 0.04	190.7 ± 10	18.6 ± 0.92	0.037 ± 0.001	19380 ± 481	1.73 ± 0.04
Pooled Within-Family Heritabilities (No Units of Measurement):						
Within-family clonal heritability ($H^2_C \pm$ se) [B]	0.31 ± 0.04	0.27 ± 0.04	0.24 ± 0.04	0.13 ± 0.02	0.45 ± 0.04	0.31 ± 0.04

[A] Phenotypic variance (σ^2_P) estimated as sum of σ^2_C, σ^2_{CT} and σ^2_e.

[B] Clonal heritability (H^2_C) is estimated as the ratio of variance between clones within-families (σ^2_C) over phenotypic variance (σ^2_P); and reflects the repeatability of clonal performance. H^2_C and σ^2_C do not include variance between-families.

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DBH (0.21 ± 0.01) and volume (0.20 ± 0.01) at 5½-years across the same series of genetic tests. DEAN *et al.* (2009) estimated substantially higher H^2_C for height (0.61 ± 0.09), DBH (0.64 ± 0.06) and volume (0.58 ± 0.08) at 7½-years across another series of four SE genetic tests in Washington and Oregon. In the case of DEAN *et al.* (2009) the parameter estimates are based on 37 SE clones propagated from four full-sib families.

(2) Stem Sinuosity: The sinuosity score of coastal Douglas-fir had a H^2_C of 0.13 ± 0.02 at 6½-years across the five Washington and Oregon tests (*Table 3*). Any confounding c-effects present in these tests may not be expected to be as serious for stem form as for growth. DEAN *et al.* (2009) reported a within-family clonal heritability $H^2_C = 0.26 \pm 0.06$ for stem sinuosity at 7½-years for SE clones in Washington and Oregon.

(3) Wood Properties: SWV had a high within-family clonal heritability of $H^2_C = 0.45 \pm 0.04$ to 6½-years across the five Washington and Oregon tests, while pilodyn had a heritability of $H^2_C = 0.31 \pm 0.04$ (*Table 3*). The site-specific within-family clonal heritability for SWV was much lower in the Washington test TH3 ($H^2_C = 0.29 \pm 0.05$) than in the other tests (values ranging from $H^2_C = 0.48$ to 0.52 ± 0.05; *Table 4*). The between-clone variance (σ^2_C) for SWV is over eight times greater than variance due to clone x test interaction (σ^2_{CT}); indicating stable ranking of clones for SWV across tests.

These H^2_C appear to be the first published estimates of genetic parameters of SE clones of coastal Douglas-fir for wood quality. CHERRY *et al.* (2008) reported narrow-sense individual heritabilities for SWV in standing open-pollinated zygotic trees (and sawn logs) of coastal Douglas-fir measured at 25-years across two sites in Washington State. The individual heritability estimated for SWV was 0.29 ± 0.09 (CHERRY *et al.,* 2008); lower than the clonal heritability reported here. As already mentioned, the H^2_C reported here are within-family estimates that do not include between-family variance in SWV.

Clonal Stability

Stability of clonal performance across tests was high to very high for all traits, with between-test correlations ranging from 0.79 ± 0.06 for stem sinuosity to 0.96 ± 0.01 for SWV (*Table 4*). These high correlations imply that clones rank similarly across each of the five tests in Oregon and Washington. A correlation of zero would imply there is no correspondence at all between rankings across the tests. DEAN (2008) and DEAN *et al.* (2009) also report stable clonal performance for growth and stem quality of somatic trees across Washington and Oregon. The between-test correlations of DEAN (2008) estimated from the same series of field tests are, as expected, very similar to the current study with 0.84 ± 0.04 for height, DBH and volume at 5½-years. The stability of genetic expression that is evident from the high between-test clonal correlations is also reflected in the low levels of variance due to clone x test interactions.

Correlations among Traits

Within families, height, DBH and volume were all strongly genetically correlated at the clonal level (correlations of 0.86 to 0.98; *Table 5*). Stem sinuosity scores had quite strong positive (unfavourable) clonal correlations with growth traits (correlations of 0.47 to 0.57; *Table 5*). These correlations indicate that clonal selection within families for growth will be accompanied by deterioration in sinuosity unless selection pressure is also applied to the stem quality trait. DEAN *et al.* (2009) also reported strong unfavourable clonal genetic correlations between growth and stem sinuosity of SE clones of coastal Douglas-fir.

Pilodyn had moderate positive (unfavourable) within-family clonal genetic correlations of 0.38 and 0.34 ± 0.11 with DBH and volume, respectively (*Table 5*). Height was less strongly unfavourably correlated with pilodyn (0.15 ± 0.12; *Table 5*). In general these clonal genetic correlations imply that faster growing SE clones tend to have higher pilodyn values, which are associated with lower wood SG. However, within-family selection of SE clones on height growth alone should have less indirect impact on pilodyn than selection on DBH or volume.

It is evident that SWV exhibited within-family clonal genetic correlations of zero or near-zero with growth and stem form (*Table 5*). These very small clonal genetic correlations suggest that it is possible to select SE clones for growth or stem form without any indirect effects on SWV. There was a small negative within-family clonal genetic correlation of –0.13 ± 0.12 between SWV and pilodyn (*Table 5*).

Table 4. – ASREML estimates of within-family clonal heritabilities and standard errors determined with fitted MODEL 2 for stem height, diameter at breast-height (DBH) and stem volume at 7½-years; and stem sinuosity, stress wave velocity (SWV) and pilodyn at 6½-years for coastal Douglas-fir somatic clones in each of the genetic tests LV1, LV2, TH3, TH4 and SP5. Overall between-test clonal correlations are also shown.

	Height (m)	DBH (mm)	Volume (dm^3)	Sinuosity (score)	SWV (m/s)	Pilodyn (mm)
LV1 (Longview, Washington)	0.38 ± 0.05	0.36 ± 0.05	0.32 ± 0.05	0.12 ± 0.03	0.52 ± 0.05	0.38 ± 0.05
LV2 (Longview, Washington)	0.38 ± 0.05	0.36 ± 0.05	0.29 ± 0.05	0.13 ± 0.03	0.48 ± 0.05	0.32 ± 0.05
TH3 (Twin Harbors, Washington)	0.37 ± 0.05	0.31 ± 0.05	0.29 ± 0.05	0.18 ± 0.04	0.29 ± 0.05	0.40 ± 0.05
TH4 (Twin Harbors, Washington)	0.41 ± 0.05	0.31 ± 0.05	0.32 ± 0.05	0.26 ± 0.04	0.52 ± 0.05	0.28 ± 0.05
SP5 (Springfield, Oregon)	0.42 ± 0.05	0.37 ± 0.05	0.36 ± 0.05	0.20 ± 0.04	0.51 ± 0.05	0.46 ± 0.05
Between-test clonal correlation	0.81 ± 0.04	0.81 ± 0.04	0.80 ± 0.04	0.79 ± 0.06	0.96 ± 0.01	0.86 ± 0.03

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Table 5. – ASREML estimates with fitted MODEL 3 of phenotypic and within-family clonal genetic correlations (and standard errors) among stem height, diameter at breast-height (DBH) and volume at 7½-years; and stem sinuosity, stress wave velocity (SWV) and pilodyn at 6½-years for coastal Douglas-fir somatic clones across tests LV1, LV2, TH3, TH4 and SP5.

	DBH	Volume	Sinuosity	SWV	Pilodyn
Within-Family Clonal Genetic Correlations:					
Height	0.86 ± 0.03	0.93 ± 0.02	0.57 ± 0.09	0.03 ± 0.13	0.15 ± 0.12
DBH		0.98 ± 0.01	0.47 ± 0.10	0.00 ± 0.13	0.38 ± 0.11
Volume			0.53 ± 0.10	0.00 ± 0.13	0.34 ± 0.11
Sinuosity				0.02 ± 0.13	0.11 ± 0.13
SWV					-0.13 ± 0.12
Phenotypic Correlations:					
Height	0.84 ± 0.01	0.87 ± 0.01	0.27 ± 0.03	0.17 ± 0.05	0.32 ± 0.04
DBH		0.95 ± 0.00	0.27 ± 0.03	0.15 ± 0.05	0.43 ± 0.03
Volume			0.29 ± 0.02	0.14 ± 0.04	0.37 ± 0.03
Sinuosity				0.04 ± 0.03	0.09 ± 0.03
SWV					0.02 ± 0.05

Conclusions

Following are the main conclusions from analyses of five genetic tests of 70 somatic clones developed from two related full-sib families of coastal Douglas-fir and measured at 6½ or 7½-years –

(1) Genetic Parameters: Within-family clonal heritabilities of growth traits at 7½-years were moderately high with low standard errors. Heritability of height was $H^2_C = 0.31 \pm 0.04$, DBH 0.27 ± 0.04 and volume 0.24 ± 0.04. Height, DBH and volume were all closely genetically correlated at the clonal level within-families. Any c-effects that may be present in this study are completely confounded with H^2_C, and these effects may lead to upward bias in the genetic parameter estimates. The H^2_C reported here are within-family estimates that do not include between-family variance.

Within-family clonal heritabilities for stem and wood quality traits at 6½-years were low in the case of stem sinuosity ($H^2_C = 0.13 \pm 0.02$) and high for SWV and pilodyn ($H^2_C = 0.45$ and 0.31 ± 0.04, respectively). Stem sinuosity and pilodyn had quite strong unfavourable clonal correlations with growth traits; while SWV had zero or near-zero clonal correlations with growth. Selection of SE clones for growth would lead to a deterioration in sinuosity and SWV unless selection pressure was applied to these stem and wood traits.

(2) Clonal Stability: Clonal performance for growth was quite stable across tests with between-test correlations of 0.80 ± 0.04 for stem volume. The between-test correlation was 0.79 ± 0.06 for sinuosity, 0.96 ± 0.01 SWV and 0.86 ± 0.03 for pilodyn. The stability of genetic expression that is evident from the high between-test clonal correlations is reinforced by generally low levels of variance due to clone x test interactions.

References

ANONYMOUS (2006): TreeSonic Microsecond Timber User's Guide Ltd, FAKOPP Enterprise Bt. H-9421 Agfalva, Fenyo u. 26., HUNGARY. (http://www.fakopp.com/site/downloads/TreeSonic_Guide.pdf)

ALLAN, J. (2003): The technology of clonal forestry of conifers, pp. 7–8. *In:* Clonal Forestry: Who are You Kidding? Abstracts Conf. Nordic Group for the Manag. Genet. Res. of Trees, edited by. T. EYSTEINSSON. Barony Castle, Scotland, September 2002. IFRS Rept. 17/2003.

BALTUNIS, B. S., D. A. HUBER, T. L. WHITE, B. GOLDFARB and H. E. STELZER (2005): Genetic effects of rooting loblolly pine stem cuttings from a partial diallel mating design. Can. J. For. Res. **35**: 1098–1108.

BENOWICZ, A., S. C. GROSSNICKLE and Y. A. EL-KASSABY (2002): Field assessment of Douglas-fir somatic and zygotic seedlings with respect to gas exchange, water relations, and frost hardiness. Can. J. For. Res. **32**: 1822–1828.

BRUCE, D. and D. J. DEMARS (1974): Volume equations for second-growth Douglas-fir. Forest Service Research Note PNW-239, USDA, PNWFRES: Portland, Oregon.

CHELIAK, W. M. and D. L. ROGERS (1990): Integrating biotechnology into tree improvement programs. Can. J. For. Res. **20**: 452–463.

CHERRY, M. L., V. VIKRAM, D. BRIGGS, D. W. CRESS and G. T. HOWE (2008): Genetic variation in direct and indirect measures of wood stiffness in coastal Douglas-fir. Can. J. For. Res. **38**: 2476–2486.

CYR, D. R., W. R. LAZAROFF, S. M. A. GRIMES, Q. Q. QUAN, T. D. BETHUNE, D. I. DUNSTAN and D. R. ROBERTS (1994): Cryopreservation of interior spruce (*Picea glauca engelmanni* complex) embryogenic cultures. Plant Cell Rep. **13**: 574–577.

DEAN, C. A. (2007): Weyerhaeuser's Douglas-fir Tree Improvement Program – Beyond 50 years, in Joint SFTIC/WFGA Conference: Tree Improvement in North America: Past, Present and Future. June 19–22, 2007. Texas: USA. (http://tfsweb.tamu.edu/conferences/treeconference/).

DEAN, C. A. (2008): Genetic parameters of somatic clones of coastal Douglas-fir at 5½-years across Washington and Oregon, USA. Silvae Genet. **57**: 269–275.

DEAN, C. A. and R. W. STONECYPHER (2006): Early selection of Douglas-fir across south central coastal Oregon, USA. Silvae Genet. **55**: 135–141.

DEAN, C. A., D. E. WELTY and G. E. HEROLD (2009): Performance and genetic parameters of somatic and zygotic

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progenies of coastal Douglas-fir at 7½-years across Washington and Oregon, USA. Silvae Genet. **58**: 212–219.

GILMOUR, A. R., B. J. GOGEL, B. R. CULLIS and R. THOMPSON (2006): ASReml User Guide Release 2.0 VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

GROSSNICKLE, S. C. and R. FOLK (2005): Stock quality assessment of a somatic interior spruce seedlot. North. J. Appl. For. **22**: 197–202.

GUPTA, P. K., R. TIMMIS, K. TIMMIS, W. CARLSON, J. GROB and E. WELTY (1994): Plantlet regeneration via somatic embryogenesis in Douglas-fir (*Pseudotsuga menziesii*), pp. 35–39 in TAPPI Proc. Bio. Science Symposium. Norcross, GA.

HARGREAVES, C. L., L. J. GRACE, D. G. HOLDEN (2002): Nurse culture for efficient recovery of cryopreserved *Pinus radiata* D. Don embryogenic cell lines. Plant Cell Reports **21**: 40–45.

HÖGBERG, K.-A. (2003): Somatic embryogenesis – A useful tool or extravagance? Pp. 12–16. *In:* Clonal Forestry: Who are You Kidding? Abstracts Conf. Nordic Group for the Manag. Genet. Res. of Trees, edited by T. EYSTEINSSON. Barony Castle, Scotland, 4–7 September, 2002. IFRS Rept. 17/2003.

HUANG, C. L. (2005): System and method for measuring stiffness in standing trees. U.S. Patent No. 6,871,545. Washington, DC: US Patent and Trademark Office.

ISIK, F., B. Li BAILIAN and J. FRAMPTON (2003): Estimates of additive, dominance and epistatic genetic variances from a clonally replicated rest of loblolly pine. Forest Science **49**: 77–88.

NAGMANI, R., M. A. JOHNSON and R. J. DINUS (1991): Effect of explant and media on initiation, maintenance, and maturation of somatic embryos in *Pseudotsuga menziesii* var. *menziesii* (MIRB.) FRANCO (Douglas-fir), pp. 171–178. *In:* Woody Plant Biotechnology. Edited by M. R. AHUJA. Plenum Press, New York.

PARK, Y. S., J. D. BARRETT and J. M. BONGA (1998): Application of somatic embryogenesis in high-value clonal forestry: Deployment, genetic control, and stability of cryopreserved clones. In Vitro Cell. Dev. Biol. Plants **34**: 231–239.

PATTERSON, H. D. and R. THOMPSON (1971): Recovery of interblock information when block sizes are unequal. Biometrika **58**: 545–54.

SEARLE, S. R., G. CASELLA and C. E. MCCULLOCH (1992): Variance Components. John Wiley and Sons: New York.

STONECYPHER, R. W., R. F. PIESCH, G. G. HELLAND, J. G. CHAPMAN and H. J. RENO (1996): Results from genetic tests of selected parents of Douglas-fir (*Pseudotsuga menziesii* [MIRB.] FRANCO) in an applied tree improvement program. For. Sci. Monograph **32**: 1–35.

SUTTON, B. (2002): Commercial delivery of genetic improvement to conifer plantations using somatic embryogenesis. Ann. For. Sci. **59**: 657–661.

Genetic Parameters and Genotype by Environment Interaction in Radiata Pine for Growth and Wood Quality Traits in Australia

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Abstract

The phenotypic response of genotypes across different environments can be quantified by estimating the genotype by environment interaction (GxE). In a practical sense, GxE means that the relative performance of genotypes does not remain constant under all test conditions. Genetic parameters and genotype by environment interactions for wood density, growth, branching characteristics and stem straightness were investigated in eight radiata pine progeny trials derived from a second generation breeding population in Australia. Five trials were on the mainland, while three trials were in Tasmania. Generally, $\hat{h}^2$ for density > branch angle > stem straightness > tree diameter > branch size; and significant $\hat{h}^2$ was observed for all traits and at all trials with only two exceptions. Genetic correlations were estimated among the five traits, and a large negative genetic correlation observed between wood density and tree diameter indicated that a selection strategy should be developed in dealing with this adverse genetic correlation in advanced generations of breeding for radiata pine.

Interactions for density, branch angle, and stem straightness were small within the two regions. Overall, branch angle had the least GxE, followed by density and stem straightness. Growth traits (tree diameter and branch size) tended to be the most interactive with substantial GxE present. Genotype by regional interactions (Mainland versus Tasmania) revealed that density and branch angle had the least interactions ($\hat{r}_B$ = 0.98 and $\hat{r}_B$ = 0.95, respectively). Branch size and tree diameter had the highest interactions among the two regions ($\hat{r}_B$ = 0.55 and $\hat{r}_B$ = 0.63, respectively). Within Tasmania, only branch size and tree diameter had a sizable interaction within the three sites. In contrast, there was little interaction for tree diameter among the Mainland

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