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Heritabilities, Intertrait Genetic Correlations, G x E Interaction and Predicted Genetic Gains for Acoustic Velocity in Mid-rotation Coastal Douglas fir

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Abstract

Acoustic velocity (AV) data from 7,423 coastal Douglas-fir trees drawn from 347 wind-pollinated families on 14 sites, from four first-generation testing programs in the north Oregon Cascades, were analyzed. Families were measured on two or four sites at ages 23 to 41 years from seed using the Fakopp TreeSonic standing-tree tool. Height (HT) and DBH data collected at ages 15 and 16 from seed, from all trees in the four programs (95,795 trees, 955 families), were used to calculate volume index ($VOL = HT \cdot DBH^2$) and stem taper ($TAP = DBH/HT$). All traits were analyzed using multivariate mixed model analyses.

Across-site individual narrow-sense heritabilities for AV^2 ranged from 0.24 to 0.40 among first-generation programs, compared to 0.12 to 0.23 for HT, 0.10 to 0.16 for DBH, 0.11 to 0.20 for VOL and 0.14 to 0.17 for TAP.

Across-site type B correlations for AV^2 ranged from 0.85 to 0.95, compared to 0.62 to 0.83 for HT, 0.60 to 0.74 for DBH, 0.67 to 0.78 for VOL and 0.66 to 0.79 for TAP. AV^2 was negatively correlated with HT in three programs ($r_A = 0.17$ to -0.28), and negatively correlated with DBH (-0.12 to -0.46), VOL (-0.05 to -0.44) and TAP (-0.09 to -0.40) in all four programs.

Selecting the top 10% of the families sampled based on AV^2 gave predicted gains of 4.4% to 9.6% for AV^2 and

–9.3% to 10.6% for VOL. The adverse genetic correlations between AV^2 and growth, and the losses in gain in AV^2 from selection based on growth, may be overestimated by suppression of slower-growing families in these older tests.

Key words: acoustic velocity, dbh, Douglas-fir, genetic correlation, genetic gain, height, heritability, taper, volume index.

Introduction

Efficient selection and breeding of forest tree species to improve wood stiffness (modulus of elasticity, MOE) requires mass screening of progeny within large replicated field tests. Until recently (~2000), the main limitation was the cost of measuring MOE on a large scale. Therefore, wood density was often used as a surrogate trait to improve wood stiffness (dynamic MOE = density x acoustic velocity²). Within the last 10 years, however, it has become possible to measure and use acoustic velocity (AV) as a surrogate for MOE. This approach has been used in Douglas-fir and other conifers (JAYAWICKRAMA, 2001; KUMAR *et al.*, 2002; CHERRY *et al.*, 2008; MATHESON *et al.*, 2008; JAYAWICKRAMA *et al.*, 2009; WEILINGA *et al.*, 2009), and is gaining acceptance as an approach to improve wood stiffness in operational breeding programs (JAYAWICKRAMA *et al.*, 2009). In fact, compared to wood density, it may be preferable to measure and select solely on AV (e.g., LI *et al.*, 2007) because it seems to have a higher correlation with static and dynamic MOE (JOHNSON and GARTNER, 2006; CHERRY *et al.*, 2008).

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The incorporation of wood stiffness into breeding programs of coastal Douglas-fir in the US Pacific Northwest (PNW) was recently reviewed by JAYAWICKRAMA *et al.* (2009). Because Douglas-fir lumber is valued for its high MOE, there is interest in counteracting the potential decreases in stiffness caused by shorter rotations and genetic selection for volume growth (JAYAWICKRAMA *et al.*, 2009). Of particular interest for the cooperative Douglas-fir tree improvement programs in the PNW are tools that can be used to measure AV on standing trees (e.g., FAKOPP TreeSonic, Fibre-Gen ST300, IML Hammer, and TreeTap).

There have been two studies on the inheritance of AV in Douglas-fir. In the first, the Fibre-Gen HM200 log tool was used to measure 39 families on four sites on the northwest Oregon coast (JOHNSON and GARTNER, 2006). In the second study, in the Puget Sound Basin, the HM200 was used to measure 129 families on two sites, and the Fibre-Gen ST300 standing-tree tool was used to measure a subset of 50 families on two sites (CHERRY *et al.*, 2008). Although the second study demonstrated that standing-tree tools can be used to indirectly and successfully select for bending stiffness (CHERRY *et al.*, 2008), it is important to measure more families on more sites, and with other standing-tree tools, to be able judge the full potential of using these tools to improve Douglas-fir wood stiffness.

The North Oregon Cascades Tree Improvement Cooperative (NOCTIC) is one of several second-generation Douglas-fir breeding cooperatives that operate in the PNW under the umbrella of the Northwest Tree Improvement Cooperative (NWTIC). NOCTIC began collecting AV data on mature trees in first-generation testing programs in 2008. This effort continued in 2009 and was supplemented by data collected as part of the Conifer Translational Genomics Network (CTGN), a multi-species research program aimed at developing the information needed to implement marker aided selection in tree breeding programs in the US. These latter data were collected in one of the Bureau of Land Management's first-generation testing programs, which is also located in the area served by NOCTIC. Overall, we report on data collected from four first-generation breeding programs in the north Oregon Cascades. Programs A and B are associated with high- and low-elevation breeding zones in the vicinity of Sweet Home, Program C is associated with a breeding zone located southeast of Portland, and Program D is associated with a breeding zone that overlaps with Program B. The field tests ranged from 23 to 41 years old from seed at the time of measurement. A total of 347 families were measured on a total of 14 test sites. Our study differed from the two previous studies of coastal Douglas-fir because we (1) tested the Fakopp TreeSonic tool, (2) sampled a different geographic area, (3) sampled a larger number of trees, sites, and families, (4) sampled one location per tree, and (5) measured AV at ages that are close to the final harvest age for coastal Douglas-fir. Furthermore, the number of families and sites we measured was considerably larger, and the trees were considerably older than in studies of other species (KUMAR *et al.*, 2002; KUMAR, 2004; KUMAR *et al.*, 2006; LI *et al.*, 2007; MATHESON *et*

al., 2008; WEILINGA *et al.*, 2009). Therefore, our genetic parameters are more robust and should provide Douglas-fir breeders a better basis on which to improve mature wood stiffness using AV measured with standing-tree tools.

Cooperative first-generation programs of Douglas-fir in the Pacific Northwest received their last complete growth measurement at age-15 or earlier. By that age, almost all important selection decisions were made, including selections for breeding orchards, production orchards, and second-cycle crossing. Therefore, a key objective was to study the relationship between growth around age-15 and stiffness of older trees. In coastal Douglas-fir, only a small proportion of the harvest-age tree (i.e., trees 35 to 60 year-old) is laid down by age-15. Therefore, foresters are keenly interested in whether early selection for growth will adversely affect mature wood quality. Corewood, which is the wood produced near the pith of the tree (and often called juvenile wood), is distinguished from outerwood by differences in wood properties, including wood stiffness. In coastal Douglas-fir, there is a gradual transition in wood properties from the corewood to outerwood that plateaus between the ages of 15 and 40, depending on the trait (ABDELGADIR and KRAHMER, 1993; PETERSON *et al.*, 2007).

Therefore, our objectives were to use mid- to late-rotation coastal Douglas-fir progeny tests to (1) estimate genetic parameters for AV, (2) understand the genetic relationships between growth traits and AV, (3) estimate predicted genetic gains for AV and growth traits under different selection scenarios, (4) develop efficient methods for measuring AV in progeny tests and estimate measurement production rates, and (5) discuss implications of findings on operational tree improvement.

Materials and Methods

Plant materials

The trees used in this study were grown from wind-pollinated seed collected from parents included in four first-generation breeding programs that are typical of Douglas-fir cooperative programs in the US Pacific Northwest (SILEN and WHEAT, 1979). For each program, a large number of parents (180 to 375) were selected from native stands within a relatively small geographic area (breeding zone), and their wind-pollinated progeny (from the wild parent trees) were tested on six to nine sites within the same zone. The parent trees were typically vigorous, reasonably well-formed trees easily accessible from a forest road, and bearing a natural cone crop. For AV, we preferentially measured families that had been selected for superior volume growth, focusing on families that had either their (1) seed parent or (2) one to several wind-pollinated progeny (forward selections) grafted into (1) production orchards and/or (2) included in second-cycle breeding. Forward selections are trees selected from the progeny tests because of their superior individual-tree and family growth rates. Some below average families were also sampled to counteract the potential bias caused by population truncation based on volume growth.

Table 1. – Overview of Douglas-fir populations sampled for acoustic velocity.

Program	Elevation range (m)	Growth traits: Numbers of			Acoustic velocity: Number of		
		Families tested	Test sites	Trees used in analysis	Families sampled	Test sites	Trees sampled
A	545-909	183	10	18,727	60	4	1,270
B	Up to 545	205	9	19,908	58	4	1,098
C	303-758	375	9	31,536	100	4	2,729
D	303-818	192	6	25,624	129	2	2,326

Table 2. – Characteristics of the 14 test sites used in this study.

Program	Site ID	Latitude	Longitude	Elevation (m)	Sowing year	Age when AV was measured	Spacing (m)	Thinned before AV assessment?
A	121217	44° 34'	122° 37'	515	1973-4	35	2.4 x 2.4	Yes
A	121224	44° 37'	122° 38'	697	1973-4	35	2.4 x 2.4	Yes
A	121319	44° 40'	122° 28'	545	1973-4	35	2.4 x 2.4	Yes
A	121326	44° 31'	122° 28'	788	1973-4	35	2.4 x 2.4	No
B	111214	44° 38'	122° 42'	424	1973	35	2.4 x 2.4	Yes
B	111215	44° 29'	122° 40'	439	1973	35	2.4 x 2.4	Yes
B	111309	44° 35'	122° 41'	273	1973	35	2.4 x 2.4	No
B	111318	44° 39'	122° 28'	545	1973	35	2.4 x 2.4	Yes
C	020201	44° 52'	122° 33'	752	1969	41	3 x 3	No
C	020301	44° 42'	122° 28'	788	1969	41	3 x 3	Yes
C	025512	45° 13'	122° 24'	394	1969	41	3 x 3	Yes
C	025515	45° 09'	122° 23'	500	1969	41	3 x 3	No
D	590702	44° 33'	122° 40'	424	1986	23	2 x 2	Yes
D	590706	44° 40'	122° 44'	364	1986	23	2 x 2	Yes

Table 1 includes information on the breeding programs used in this study, including the elevation range of each breeding zone, number of families tested, number of test sites, and numbers of families and trees measured for growth and acoustic velocity. Within each first-generation breeding zone, two or four test sites were measured, focusing on sites with high survival, high heritabilities for growth traits (age 15 or 16), and the ability to easily identify the trees using plantation maps and tree tags. These test plantations were established using either a “reps-in-sets” or “sets-in-reps” design. In the “reps-in-sets” design, the blocks (or replicates) of each set were planted adjacent to one another on the site, and each set is typically treated as a separate experiment. In the less common “sets-in-reps” design, the sets were randomized within blocks. For both designs, families were planted in three- or four-tree non-

contiguous plots at each site. The characteristics of the test sites are described in *Table 2*. Overall, we measured 58 families in Program A and 60 families in Program B between mid-August and late September 2008; 100 families in Program C in July 2009; and 129 families in Program D in August 2009.

Measurements

All living trees from the selected families were measured for acoustic velocity (AV) using a Fakopp TreeSonic device (Fakopp Enterprise, Hungary), except trees with broken tops, severe stem damage in the lower bole, and dubious identity. Because moisture content of the wood is known to affect AV and stiffness, all AV measurements were taken in late summer and early fall when the moisture contents of the trees are lowest (i.e., because western Oregon has a Mediterranean climate

with a marked summer drought). Although we assumed that this would give better results than a time when moisture content was changing rapidly (e.g., late spring), recent work in radiata pine suggests that variation in wood moisture content has only a small affect on variation in acoustic velocity (WEILINGA *et al.*, 2009b). Nonetheless, late summer is also a time when measurement crews are generally available and when AV equipment is unlikely to be damaged by rain or snow.

On each sampled tree, the start sensor of the TreeSonic was driven into the stem at about 2.0 m, and the stop sensor was placed directly below it at about 1.0 m. The height was sometimes varied to avoid large clusters of branches or wounds on the stem, but the distance between the probes was kept constant at 1.0 m. The sensors were placed at opposing 45° angles to the stem on the same side of each tree (i.e., same azimuth). Further details on the TreeSonic are provided in the TreeSonic user manual available on the manufacturer's website (www.fakopp.com).

Acoustic waves were induced by striking the start sensor with a hammer, and the transit time (μs) between the start and stop sensors was recorded. Three consecutive readings were taken at the same location on each tree and then averaged to yield the final transit time (TIME), which was then used to calculate squared acoustic velocity AV² in km²/s² as (1000/TIME)². Because a preliminary study had shown that taking a second measurement on the same tree took almost as long as measuring a second tree, we decided to measure more trees, rather than take multiple measurements per tree. In radiata pine, variation in acoustic velocity between the sides of a tree was only 4.3% of total variation at age 10 and 8.5% at age 15 (TOULMIN and RAYMOND, 2007). Furthermore, the correlations between measurements on one side versus the mean of four sides were 0.98 and 0.96 at ages 10 and 15 respectively (TOULMIN and RAYMOND, 2007). Tree height (HT, cm) and diameter at breast height (DBH, mm) were measured at age 15 and used to calculate tree volume index (VOL = HT x DBH²) and taper (TAP = DBH/HT).

Statistical analyses

Single-site analysis

Each program was analyzed using the following single-site statistical model for each trait:

$$y_{ijkl} = \mu + \alpha_i + R_j + \tau_k + (\tau R)_{jk} + e_{ijkl} \quad (1)$$

where y_{ijkl} is the l th observation from the k th family in the j th replicate and i th set, μ is the population mean, α_i is the effect of the i th set, R_j is the effect of the j th replicate, τ_k is the effect of the k th family, $(\tau R)_{jk}$ is the interaction of the j th replicate and k th family, e_{ijkl} is the residual error. Replicate effects were estimated differently and have different interpretations, within sites for the “sets-in-reps” and within sets and sites for the “reps-in-sets” designs. All effects were considered random except for μ and α_i . The assumptions for the random effects are τ_k are iid $N(0, \sigma_{\tau}^2)$, R_j are iid $N(0, \sigma_R^2)$, $(\tau R)_{jk}$ are iid $N(0, \sigma_{\tau R}^2)$, e_{ijkl} are iid $N(0, \sigma_e^2)$, and the effects of τ_k , R_j , $(\tau R)_{jk}$, and e_{ijkl} are independent. Variance compo-

nents were estimated by the Restricted Maximum Likelihood (REML) method using ASReml (GILMOUR *et al.*, 2006).

We assumed that the family variance components estimated one-quarter of the additive genetic variance (FALCONER and MACKAY, 1996), and then estimated heritabilities for individual trees (h_i^2) and family means (h_f^2) for each site using the following equations:

$$h_i^2 = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_{fr}^2 + \sigma_e^2} \quad (2)$$

and

$$h_f^2 = \frac{\sigma_f^2}{\sigma_f^2 + \frac{k_2}{k_1} \sigma_{fr}^2 + \frac{1}{k_1} \sigma_e^2} \quad (3)$$

where σ_f^2 , σ_{fr}^2 and σ_e^2 are the variance components for family, family x replicate, and residual, respectively, and k_1 and k_2 are the component coefficients based on approximate stratum variance decomposition. Approximate standard errors of the heritabilities were calculated using the delta method (LYNCH and WALSH, 1998) based on the matrices of variances and covariances estimated via ASReml. These approximate standard errors should be used with caution because their distributions are unknown (FALCONER and MACKAY, 1996).

Multi-site analysis

We conducted single-trait and multi-trait analyses by fitting a mixed model to the data combined across test sites. Data were first standardized by dividing each observation by the phenotypic standard deviation of the corresponding site to remove scale effects. The general linear mixed model used was:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_r\mathbf{r} + \mathbf{Z}_f\mathbf{f} + \mathbf{Z}_{fs}\mathbf{fs} + \mathbf{e} \quad (4)$$

where $\mathbf{y} = [\mathbf{y}_1' \mathbf{y}_2' \dots \mathbf{y}_t']'$ is the vector of observations for the t traits for each individual, $\mathbf{b} = [\mathbf{b}_1' \mathbf{b}_2' \dots \mathbf{b}_t']'$ is the vector of fixed effects for the overall mean, site, set, and site x set interaction, $\mathbf{r} = [\mathbf{r}_1' \mathbf{r}_2' \dots \mathbf{r}_t']'$ is the vector of random replicate-within-site effects, $\mathbf{f} = [\mathbf{f}_1' \mathbf{f}_2' \dots \mathbf{f}_t']'$ is the vector of random family effects, $\mathbf{fs} = [\mathbf{fs}_1' \mathbf{fs}_2' \dots \mathbf{fs}_t']'$ is the vector of random family x site effects, and $\mathbf{e} = [\mathbf{e}_1' \mathbf{e}_2' \dots \mathbf{e}_t']'$ is the vector of random residuals. $\mathbf{X}$, $\mathbf{Z}_r$, $\mathbf{Z}_f$ and $\mathbf{Z}_{fs}$ are the corresponding incidence matrices. Replicate effects were estimated differently and have different interpretations for the “sets-in-reps” design and “reps-in-sets” designs. We assumed that the random effects were uncorrelated and distributed as multivariate normal variables with zero means and a variances-covariances structure as described below:

$$\mathbf{V} \begin{bmatrix} \mathbf{r} \\ \mathbf{f} \\ \mathbf{fs} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{G}_r \otimes \mathbf{I} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G}_f \otimes \mathbf{I} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{G}_{fs} \otimes \mathbf{I} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{R} \otimes \mathbf{I} \end{bmatrix} \quad (5)$$

where 0 is a null matrix, I is an identity matrix, $\otimes$ is the Kronecker product. $\mathbf{G}_r = \{\sigma_{r_i r_j}\}$, $\mathbf{G}_f = \{\sigma_{f_i f_j}\}$, $\mathbf{G}_{fs} = \{\sigma_{fs_i fs_j}\}$, and $\mathbf{R} = \{\sigma_{e_i e_j}\}$ are the replicate-within-site, family, family x site, and residual covariance matrices, respectively.

For variance components, $\sigma_{r_i r_j}^2 = \sigma_r^2$, $\sigma_{f_i f_j}^2 = \sigma_f^2$, $\sigma_{f_s f_s_j}^2 = \sigma_{f_s}^2$, and $\sigma_{e_i e_j}^2 = \sigma_e^2$. The models were fitted for each program using the REML method and ASReml software. For the single-trait analyses, we assumed that there was a common additive genetic variance across sites and site-specific residual variances. The primary purpose of these analyses was to predict across-site breeding values for individual traits. Genetic gains were then predicted for each parent and progeny by dividing its predicted breeding value by the estimated population mean. The primary purpose of the multi-trait analyses was to estimate across-site genetic parameters such as heritabilities and genetic correlations. Rather than using only the trees that had been measured for AV², these analyses included all trees in the program that had been measured for HT, DBH, VOL or TAP because the resulting correlations among growth traits are more reliable than those based on the small subset of trees sampled for AV. Given the variance components estimated from the single-trait analyses, the heritabilities were estimated as:

$$h_i^2 = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_{f_s}^2 + \sigma_e^2} \quad (6)$$

and

$$r_G = \frac{\sigma_{f_A f_B}}{\sigma_{f_A} \cdot \sigma_{f_B}} \quad (7)$$

where h_i^2 and h_f^2 are the estimates of individual-tree and family-mean heritabilities, and k_1 and k_2 are the component coefficients based on approximate stratum variance decomposition. The genetic correlation between traits A and B was estimated as

$$r_G = \frac{\sigma_{f_A f_B}}{\sigma_{f_A} \cdot \sigma_{f_B}} \quad (8)$$

The Type B genetic correlation for each trait was estimated as (YAMADA, 1962):

$$r_B = \frac{\sigma_f^2}{\sigma_f^2 + \sigma_{f_s}^2} \quad (9)$$

Standard errors of the heritabilities and genetic correlations were estimated using the delta method (LYNCH and WALSH, 1998) based on the matrices of variances and covariances estimated via ASReml.

BLUP breeding values were estimated by incorporating the numerator relationship matrix based on pedigree information. Genetic gains were predicted for each parent and progeny by dividing each predicted breeding value by the estimated population mean. The predicted gains were used to explore various selection scenarios, including selection based solely on VOL, HT, or AV². We also explored gains using various weights on VOL and AV² for Programs C and D, which had the highest and second-highest numbers of families sampled. We used weights of 0.0 to 1.0 for VOL, and 1.0 to 0.0 for AV², similar to the approach used by LI et al. (2007). Because the maximum gains for VOL were approximately five times the maximum gains for AV², the weighting was implemented by multiplying the gains for VOL by 0 to 1 (in steps of 0.1), and gains for AV² by 5 to 0 (in steps of -0.5).

The typical procedure in the operational Douglas-fir program has been to use AV² for analysis and ranking instead of AV, since AV² is more closely related to stiffness. Using AV is another option, but AV is expected to function like a square-root transformation of the variable AV². Statistically, square-root transformation on a continuous variable is often useful for reducing positive skewness, but is expected to have little effect on normally-distributed variables such as AV². For verification, heritabilities, genetic correlations and predicted gains

Table 3. – Within-site genetic parameters for the 14 test sites.

Program	Site ID	Number of trees (N)				HT				DBH				VOL				N	Velocity ²			
		HT	DBH	VOL	Individual-tree heritabilities		Family-mean heritabilities		Individual-tree heritabilities		Family-mean heritabilities		Individual-tree heritabilities		Family-mean heritabilities		Individual-tree heritabilities		Family-mean heritabilities			
					h ²	SE (h ²)	h ²	SE (h ²)	h ²	SE (h ²)	h ²	SE (h ²)	h ²	SE (h ²)	h ²	SE (h ²)	h ²		SE (h ²)	h ²	SE (h ²)	h ²
A	121217	2,333	2,329	2,328	0.3	0.19	0.48	0.16	0.3	0.19	0.43	0.18	0.3	0.20	0.4	0.17	229	0.5	0.32	0.3	0.17	
A	121224	2,015	2,012	2,011	0.4	0.18	0.46	0.11	0.3	0.20	0.43	0.16	0.3	0.20	0.4	0.16	210	0.0	0.00	0.0	0.00	
A	121319	2,360	2,354	2,353	0.3	0.17	0.47	0.16	0.3	0.17	0.47	0.12	0.4	0.20	0.5	0.13	229	0.6	0.28	0.4	0.13	
A	121326	2,351	2,326	2,325	0.1	0.11	0.24	0.14	0.1	0.12	0.18	0.20	0.1	0.13	0.2	0.18	430	0.5	0.20	0.5	0.10	
B	111214	2,056	2,057	2,055	0.2	0.15	0.39	0.17	0.2	0.16	0.35	0.20	0.2	0.16	0.3	0.19	242	0.3	0.27	0.2	0.18	
B	111215	2,080	2,075	2,075	0.1	0.14	0.29	0.20	0.1	0.12	0.25	0.17	0.1	0.13	0.2	0.16	268	0.0	0.22	0.0	0.23	
B	111309	1,873	1,870	1,870	0.1	0.12	0.20	0.15	0.1	0.15	0.28	0.17	0.2	0.12	0.2	0.11	494	0.5	0.17	0.5	0.09	
B	111318	2,057	2,055	2,051	0.1	0.10	0.20	0.13	0.1	0.13	0.29	0.16	0.1	0.13	0.2	0.16	266	0.1	0.25	0.1	0.22	
C	020201	4,284	4,275	4,272	0.1	0.13	0.30	0.15	0.1	0.13	0.26	0.19	0.1	0.13	0.2	0.18	731	0.3	0.14	0.4	0.11	
C	020301	3,614	3,591	3,544	0.1	0.14	0.25	0.15	0.1	0.15	0.22	0.20	0.1	0.15	0.2	0.18	408	0.2	0.23	0.2	0.15	
C	025512	4,197	4,186	4,184	0.2	0.15	0.35	0.16	0.2	0.16	0.35	0.20	0.2	0.17	0.3	0.20	643	0.7	0.17	0.5	0.07	
C	025515	4,250	4,250	4,249	0.1	0.15	0.32	0.19	0.1	0.12	0.24	0.17	0.1	0.13	0.2	0.16	947	0.4	0.12	0.5	0.08	
D	590702	4,379	4,379	4,379	0.3	0.14	0.60	0.11	0.3	0.14	0.59	0.12	0.3	0.15	0.6	0.11	1203	0.3	0.10	0.5	0.07	
D	590706	4,106	4,106	4,106	0.2	0.13	0.53	0.13	0.3	0.13	0.53	0.13	0.3	0.14	0.5	0.12	1123	0.4	0.12	0.4	0.08	

Table 4. – Across-site heritabilities and type B genetic correlations for the four testing programs.

Program	Trait	Individual-tree heritabilities		Family-mean heritabilities		Type B genetic correlations	
		$h^2_{(i)}$	SE_ $h^2_{(i)}$	$h^2_{(f)}$	SE_ $h^2_{(f)}$	r_B	SE_ r_B
A	HT	0.230	0.026	0.823	0.018	0.812	0.050
A	DBH	0.183	0.022	0.774	0.023	0.737	0.055
A	TAP	0.170	0.021	0.766	0.024	0.804	0.064
A	VOL	0.205	0.024	0.792	0.021	0.735	0.050
A	AV ²	0.393	0.110	0.741	0.071	0.846	0.186
B	HT	0.118	0.001	0.683	0.008	0.616	0.070
B	DBH	0.117	0.001	0.681	0.007	0.602	0.069
B	TAP	0.135	0.001	0.721	0.007	0.657	0.077
B	VOL	0.133	0.001	0.711	0.007	0.671	0.067
B	AV ²	0.242	0.010	0.629	0.029	0.926	0.268
C	HT	0.129	0.013	0.727	0.022	0.765	0.089
C	DBH	0.099	0.011	0.660	0.027	0.726	0.079
C	TAP	0.136	0.014	0.726	0.022	0.792	0.071
C	VOL	0.111	0.012	0.688	0.025	0.766	0.078
C	AV ²	0.394	0.074	0.732	0.046	0.930	0.105
D	HT	0.186	0.021	0.806	0.020	0.828	0.045
D	DBH	0.161	0.020	0.771	0.024	0.761	0.049
D	TAP	0.167	0.020	0.777	0.023	0.766	0.048
D	VOL	0.183	0.021	0.791	0.022	0.777	0.046
D	AV ²	0.398	0.079	0.652	0.066	0.954	0.141

Table 5. – Between-trait genetic correlations (below diagonals) and their standard errors (above diagonals) for the four testing programs.

Program	Trait	HT	DBH	TAP	VOL	AV ²
A	HT	–	0.019	0.087	0.013	0.165
A	DBH	0.882	–	0.058	0.004	0.178
A	TAP	0.131	0.580	–	0.071	0.193
A	VOL	0.928	0.980	0.444	–	0.175
A	AV ²	-0.195	-0.213	-0.090	-0.261	–
B	HT	–	0.025	0.091	0.015	0.166
B	DBH	0.873	–	0.043	0.005	0.170
B	TAP	0.361	0.768	–	0.060	0.191
B	VOL	0.933	0.981	0.643	–	0.159
B	AV ²	-0.284	-0.461	-0.492	-0.438	–
C	HT	–	0.030	0.076	0.020	0.131
C	DBH	0.788	–	0.032	0.005	0.128
C	TAP	0.217	0.769	–	0.047	0.136
C	VOL	0.876	0.975	0.631	–	0.123
C	AV ²	-0.267	-0.341	-0.239	-0.388	–
D	HT	–	0.029	0.084	0.019	0.139
D	DBH	0.805	–	0.044	0.004	0.141
D	TAP	0.134	0.695	–	0.058	0.123
D	VOL	0.888	0.979	0.552	–	0.138
D	AV ²	0.167	-0.117	-0.396	-0.048	–

were calculated using both AV and AV² in program D. We also calculated VOL gains foregone, which is equal to the VOL gains (VG) resulting from direct selection on VOL minus the volume gains resulting from indirect selection on AV² (i.e. VOL gain selecting on VOL – VOL gain selecting on AV²).

Results

Narrow-sense heritabilities for AV² ranged from 0.00 to 0.70 within sites (*Table 3*) and 0.24 to 0.40 across sites within first-generation programs (*Table 4*). The heritabilities for the growth traits (HT, DBH, VOL, TAP) were mostly lower, ranging from 0.11 to 0.45 within sites, and 0.10 to 0.23 across sites (*Table 4*). Family-mean heritabilities for AV² were similar to those for the growth traits in Program C, slightly lower (i.e., ~0.02 to 0.08 lower) for AV² than the growth traits in Program A and B, and moderately lower (i.e., ~0.12 to 0.15 lower) for AV² in Program D (*Table 4*). These differences are related to the inherent heritability of the trait and the

sample size, both of which differed between AV² and the growth traits. Analyses of AV and AV² in Program D resulted in a perfect across-site genetic correlation (1.0) between these traits, and similar heritabilities (0.38 versus 0.39–0.41), but different predicted gains. Therefore, it should be possible to directly compare our results for AV² with results from other studies that analyzed AV for genetic parameters, but not for predicted gain.

Across-site type B correlations ranged from 0.85 to 0.95 for AV² compared to 0.60 to 0.83 for the growth traits (*Table 4*). The genetic correlations between AV² and the growth traits were weakly to moderately negative (–0.05 to –0.46) for all growth traits and programs except for one (i.e., $r_G = 0.17$ between AV² and HT in Program D) (*Table 5*).

Selecting the top 10 % of families based on AV² resulted in predicted gains of 4.4 % to 9.6 % for AV², –1.2 % to 5.6 % for HT, and 9.3 % to 10.6 % for VOL (*Table 6*). The VOL gains foregone by selecting the top 10 % of parents based on AV² are shown in *Table 6*. Results of varying

Table 6. – Predicted percentage gains or losses in AV² and growth traits from selecting the top 10% of parents based on progeny performance across all sites in each of four programs. Except where ranges are reported, all values are the mean of separate gain estimates from sets of parents tested in each program.

Trait and scenario	Predicted gains in %, by program			
	A	B	C	D
Range of AV ² gains	11.21 to -9.61	7.17 to -9.22	15.08 to -12.72	11.74 to -12.05
AV ² gains (losses) from selecting				
Based on descending AV ² gains	9.56	4.39	8.47	7.80
Based on descending HT gains	(0.84)	(2.52)	0.33	1.72
Based on descending VOL gains	(0.48)	(2.09)	(0.19)	(1.98)
Gains (losses) from selecting based on descending AV ² gains				
HT gains (losses)	6.39	1.34	(1.24)	5.58
VOL gains (losses)	(1.06)	2.12	(9.34)	10.62
VOL gains from selecting based on VOL	92.19	39.38	61.10	44.14
¹ Potential VOL gains foregone by selecting on AV ² vs selecting on VOL	93.25	37.26	70.44	33.52
² Potential AV ² gains foregone by selecting on VOL vs selecting on AV ²	10.04	6.48	8.66	9.78

• Only the subset of parents sampled for AV were considered in these calculations.

¹ Vol gains from selecting on VOL-VOL gain (or loss) from selecting on AV².

² AV² gains from selecting on AV²-AV² gain (or loss) from selecting on VOL.

Table 7. – Correlations between predicted gains for AV² and parent tree location variables, for the four testing programs.

Program	Parent latitude	Parent departure	Parent elevation
A	0.02	-0.30	-0.05
B	-0.16	0.11	0.13
C	-0.17	-0.06	0.05
D	-0.01	0.03	0.05

the selection weights on VOL and AV² for Programs C and D are shown in *Figure 1*. Correlations between AV² and parent-tree location variables (latitude, longitude, and elevation) were weak ($|r| \leq 0.3$) (*Table 7*).

Discussion

The genetic parameters in this study were generally consistent from program to program, and quite similar to those estimated in previous studies of Douglas-fir and other species. Height was more heritable than DBH in three of the four programs, and the heritabilities for these traits were about the same as previously reported (JOHNSON *et al.*, 1997; HOWE *et al.*, 2006). AV² was 1.5 to 4 times as heritable as HT, DBH, VOL, and TAP, which is consistent with previous studies of acoustic velocity measured using standing-tree and log tools (JOHNSON and GARTNER, 2006; LI *et al.*, 2007; CHERRY *et al.*, 2008; WEILINGA *et al.*, 2009). Because of within-tree variation in wood properties, heritabilities may differ between acoustic velocity measured with a log tool (such as the HM200) and a standing-tree tool (such as the TreeSon-

ic). Although they did not test a standing-tree tool, JOHNSON and GARTNER (2006) reported an across-site heritability of 0.48 using the HM200 on Douglas-fir. Furthermore, this heritability would have been about 0.64 if they had estimated the additive genetic variance as $4\sigma_f^2$ as we did, instead of $3\sigma_f^2$. In contrast, CHERRY *et al.* (2008) reported nearly equal across-site heritabilities for the HM200 log tool (0.30) and the ST300 standing-tree tool (0.29). Again, these two heritabilities would have been about 0.40 if they had estimated the additive genetic variance as $4\sigma_f^2$.

In radiata pine, the heritabilities for stiffness seem to be high in the first few growth rings, but decline further from the pith (DUNGEY *et al.*, 2006; KUMAR *et al.*, 2006). Standing-tree tools such as the TreeSonic mostly sample the outer few centimeters of wood, which means that we measured acoustic velocity in wood ranging from about 20 to 35 rings from the pith. Despite this range in ring age, and individual-tree heritabilities that ranged from 0.17 to 0.70 across 12 of 14 sites, we found no indication that heritabilities are lower in the outer growth rings of Douglas-fir. For example, the across-site heritabilities in our 41 year-old trial were no lower than in our 23 year-old trial, or in the 25-year old trial reported by CHERRY *et al.* (2008).

Because the individual-tree heritabilities for AV² are higher than they are for the growth traits, fewer trees need to be measured to obtain comparable family-mean heritabilities. When we measured four sites in Programs A-C, we obtained family-mean heritabilities for AV² that were similar to those for growth by measuring AV² on only one quarter as many trees. However, when we measured AV² on two sites in Program D, the family-mean heritabilities were lower for AV² than for the growth

traits that were measured on six sites. Although the numbers of families measured for the growth traits and AV^2 differed, these results were generally consistent with results from our deterministic simulation done prior to data collection. We also found that measuring one location per tree yielded heritabilities similar to those reported in other studies of standing-tree tools, including studies that measured acoustic velocity at two locations per tree (KUMAR, 2004; CHERRY *et al.*, 2008; MATHESON *et al.*, 2008) and one location per tree (KUMAR *et al.*, 2002; LI *et al.*, 2007). Information on how heritabilities are affected by the number of measured sites has been integrated into recommendations for incorporating acoustic velocity into operational breeding programs of Douglas-fir (discussed below).

Our analyses indicate that TAP is as heritable as HT, DBH, and VOL. Genetic parameters for taper may be important because taper appeared to be useful as a predictor of dynamic modulus of elasticity in *Cryptomeria japonica* (KII *et al.*, 2003). KING (1992) reported a narrow-sense heritability for stem taper of 0.10 and a family-mean heritability of 0.57, whereas JOHNSON and GARTNER (2006) reported similar heritabilities for taper and height in Douglas-fir. Stem taper appeared to be under strong genetic control in jack pine (MAGNUSSEN and KEITH, 1990) and in *Eucalyptus grandis* (VAN WYK, 1990).

Based on the type-B correlations, there is little genotype $\times$ site interaction for AV^2 . The type-B correlations were very high for AV^2 (0.84 to 0.95), and mostly high in other studies of Douglas-fir (0.85; JOHNSON and GARTNER, 2006), loblolly pine (0.68; LI *et al.*, 2007), and radiata pine (0.45 to 1.12; KUMAR, 2004; MATHESON *et al.*, 2008; WEILINGA *et al.*, 2009). Type B correlations for growth traits were lower (0.60 to 0.83), but fairly strong in our study and in a broader study of genotype $\times$ site interactions for height growth in six Douglas-fir first-generation breeding programs ($r_B = 0.72$; JOHNSON *et al.*, 1997).

Despite the high r_B , measuring only one or few sites carries risks. If trees are measured at one site $V_{F \times S}$ is confounded with V_F and the “true” family-mean heritability is essentially equal to $r_B \times h_F^2$. With an r_B value of 0.85, family-mean heritability will be over-estimated by about 15% if h_F^2 is estimated from a single site. The fewer the number of sites or trees that are measured, the higher the likelihood of unusual estimates. For example, AV^2 always had a much higher standard error than the growth traits, and we observed near zero heritabilities for AV^2 for two of our 14 sites (Table 4). The main cause of a zero-heritability could be the sample size we used, and similar zero-heritability situations occurred for the growth traits as well. For example, heritability estimates for DBH at sites 111214 and 121217 were 0.231 and 0.326 using all trees (Table 3), but were near zero when we used only the subset of families and trees that were sampled for AV.

AV^2 had generally negative (adverse) genetic correlations with the growth traits, and these adverse correlations were mostly stronger for DBH and VOL than for HT (apart from program D), which is consistent with

results from other studies (JOHNSON and GARTNER, 2006; LI *et al.*, 2007; CHERRY *et al.*, 2008). Taper had an adverse (and mostly moderate) genetic correlation with AV^2 in all four programs. The genetic correlation between acoustic velocity and taper was moderately negative (−0.44) in the study of JOHNSON and GARTNER (2006), but weakly negative (0.0 to −0.23) in another study of Douglas-fir (CHERRY *et al.*, 2008). It has been noted that cylindrical trees (i.e., trees with less taper) may need stiffer wood to avoid buckling (Mike Watt and John Moore, personal communication; cited in JOHNSON and GARTNER, 2006). Taper appeared to be useful as a predictor of dynamic modulus of elasticity in *Cryptomeria japonica* (KII *et al.*, 2003).

The exact reason why the correlation of AV^2 with height is weaker than with dbh is not known. The growing understanding that acoustic velocity is negatively correlated with diameter growth and taper is leading to a revision of selection criteria for Douglas-fir in the PNW to limit losses in stiffness, while ensuring that breeding populations have an adequate mix of genotypes with superior height growth and volume growth. This approach is designed to limit adverse changes in stem taper that may be associated with lower wood stiffness and lower log value. Because logs in the PNW are scaled using the Scribner scale, low-taper logs obtain higher prices. Because we routinely measure height in our Douglas-fir progeny tests, it is easy to increase the emphasis on height.

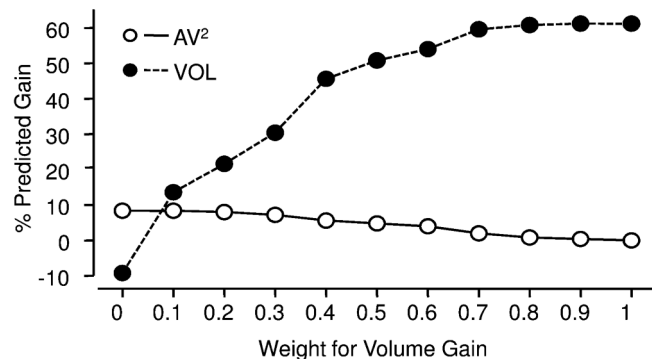
Predicted gains in AV^2 from selecting the top 10% of parents were nearly 10% in one of the four programs (range = 4.4 to 9.6%). Predicted gains of 4.1% to 24% have been reported from other studies (reviewed in JAYAWICKRAMA *et al.*, 2009), but some of these gains were based on higher selection intensities than we used in our study. Our gains in AV^2 were much lower than the potential gains for VOL, but should be considered in context with the considerations given below.

First, AV^2 was measured on a subset of parents, so some high- AV^2 parents would have been omitted. Organizations for whom stiffness is the highest priority could measure a higher proportion of families. Second, because gains in growth traits were predicted for ages 15 and 16, the gains at rotation age may be considerably less (e.g., GOULD and MARSHALL, 2009). JOHNSON *et al.* (1997) devised an age-age correlation equation of $r = 1.077 + 0.309 \times (\log \text{ of age ratio})$ for height, which would result in a correlation of 0.70 between trees at age 15 versus 50. In contrast, our gains for AV^2 were predicted at ages 23 to 41, closer to rotation age. Third, because gains in AV^2 are only for the region near breast height, we expect them to be imperfectly correlated with gains in whole-tree AV^2 . Stiffness and other wood properties vary substantially within the tree, including variation from pith-to-bark, variation along the stem, and variation among trees of different ages (reviewed in JAYAWICKRAMA *et al.*, 2009). For example, the genetic correlation between bending stiffness and wood density of a disc cut from the base of the tree was substantially different from the correlation between bending stiffness and mean lumber density (CHERRY *et al.*, 2008). Fourth,

gains in whole-tree bending stiffness will be less than gains in AV^2 because the correlation between these traits may be considerably less than one. For example, the genetic and phenotypic correlations between standing tree acoustic velocity and bending stiffness of the first 9' log were 0.53 and 0.35 in a previous study of Douglas-fir (CHERRY *et al.*, 2008). *Fifth*, if one started (as suggested by JAYAWICKRAMA *et al.*, 2009) with the goal of meeting the MOE standard of No. 2 visually graded lumber (1.6×10^6 psi), a 10% increase in stiffness would raise the average by one grade to a No. 1 grade. However, if we started with a 1600f MSR grade, this same increase in stiffness would potentially increase the MSR grade by six or seven grades.

The large differences in potential gains for AV^2 versus VOL are in contrast to the observation that proportional gains in wood density may be comparable to genetic gains in growth traits (HOWE *et al.*, 2006). Our understanding of the appropriate economic weights for Douglas-fir traits, such as VOL and AV^2 , is less advanced than in other species such as radiata pine and eucalypts, and this hinders optimal breeding for stiffness. Volume was by far the major determinant of wood value for visually graded ($R^2=0.89$) and MSR lumber ($R^2=0.83$) in one study of Douglas-fir (AUBRY *et al.*, 1998). However, because of the long time-horizon for tree breeding, the relative value of volume growth versus wood stiffness may change by the time improved trees are harvested.

Program C



Program D

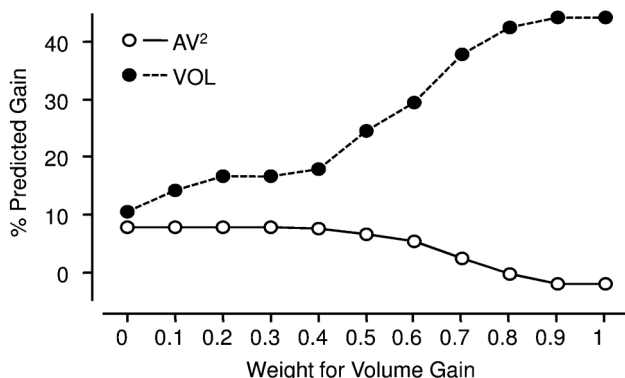


Figure 1. – Variation in predicted gain from selecting 10% of the sampled parents, with weight for predicted volume gain.

As observed in other studies, it will not be possible to simultaneously obtain maximum gains for growth traits and acoustic velocity (Figure 1). Maximum gains for growth will generally be associated with a slight loss in acoustic velocity, whereas maximum gains in acoustic velocity will be associated with a large decrease in volume gain. Nonetheless, using appropriate weights for the two traits (such as 1.5 and 0.7 for AV^2 and VOL, respectively), it may be possible to simultaneously obtain near maximum gains for VOL and positive gains for AV^2 . Therefore, selecting for some combination of VOL and AV^2 seems both feasible and desirable.

We do not know how family rankings for AV^2 are affected by branches, knots, and growing space. Because the branches near breast height (where we collected our AV data) had either died or been pruned off many years ago, and because the acoustic waves seem to travel through the outermost rings of the tree, we assume that we mostly measured clearwood. Although heavy stocking may reduce knot size and increase the proportion of clearwood measured, the slower-growing families may be preferentially suppressed, leading to smaller growth rings, denser wood, and higher stiffness in their outer wood. Thus, the negative correlation between AV^2 and growth (especially DBH) may be inflated, and the loss in AV^2 from selection for growth may be overestimated. The least adverse correlations between AV^2 and growth were observed in program D, which had the youngest trees. Furthermore, because the sites in Program D had been thinned shortly after they were measured at age 16, the trees would have been less suppressed than in the other three programs. However, a higher proportion of families were measured in program D, and that may have impacted the correlations as well.

We found little evidence that the gains in AV^2 would be influenced by the origin of the parent trees, at least for latitude, longitude, or elevation within the first-generation breeding zones. These zones span small north-south and east-west distances, but extend over considerable elevational ranges.

Implementation of acoustic velocity assessment for coastal Douglas-fir

This topic has been discussed in some length in JAYAWICKRAMA *et al.* (2009). Our results indicate that it is effective to measure AV in coastal Douglas-fir tests of this age using a sampling scheme similar to the one we used in this study. That is, by measuring AV at one location per tree on a total of 20–25 trees per family on three to four sites. Measuring AV in mid-summer works well biologically and practically because crossing is completed in the spring, and routine growth and form measurements begin in the fall after the cessation of height growth. In the PNW, there remain many first-generation progeny tests, corresponding seed orchards, and seedlots from those orchards that could be used for improving acoustic velocity over the next five years or so. Those data could be quickly translated to gain in operational plantations.

Extension to younger (e.g., 10- to 15-year-old) second-cycle tests will be the next step to consider. In second-

cycle tests, breeders must deal with several challenges: small-diameter trees (including trees that may be unmeasurable), boles with a larger proportion of live branches and knots, and higher moisture contents in the wood. Some verification that AV is still an acceptable predictor of bending stiffness in these smaller standing trees is required. One advantage is that probe insertion should be easier in the softer, moister wood.

We measured 7,423 trees at an average rate of 78 trees per 10-hr person-day. In good conditions, the production rate increased to 100 trees per person-day, and could be higher still. Using the IML Hammer, MATHESON *et al.* (2008) reported a rate of 90 trees per hour for a three-person crew (equivalent to 210 trees per person-day, assuming a 7-hr measurement day). Other production estimates include a rate of 420 trees per person-day in slash pine (HUBER *et al.*, 2006), and 400 trees per person-day using the TreeSonic in loblolly pine (JAYAWICKRAMA *et al.*, 2009). Daily production rates can be increased by short travel distances; well-trained crews; systematic, simple thinning patterns (or unthinned tests); prior pruning of the site; and by sampling a high proportion of trees on the site. One challenge is that the mature wood of Douglas-fir, especially when tightly grown, is very dense and resists the entry of the TreeSonic probes. By pre-drilling the holes with an electric drill, it may be possible to reduce the fatigue caused by repeated pounding of the probes. Although the TreeSonic tool worked well, there are other standing-tree tools that may work as well.

Selection based on a log tool such as the HM200 will give higher gains in bending stiffness compared to a standing-tree tool, since the log tool gives a better prediction of bending stiffness than the standing-tree tool (e.g., KUMAR *et al.*, 2002; CHERRY *et al.*, 2008). Although log tools have gained wide acceptance for sorting logs at landings or in log yards, the production rates in operational forestry cannot be obtained when a log tool is used in progeny testing because of the additional steps that are needed. The extra steps in progeny testing include labeling the logs prior to harvest, finding logs after harvest, and checking the labels at the time of measurement. It is necessary to measure log lengths when using the log tool, and this typically takes longer than a short one meter measurement for the standing tree tool. The log tool does have an advantage over the standing tree tool in that it is not necessary to drill holes or insert probes, so the actual data collection step can be much faster. Despite that advantage the overall production rate with a log tool may be one-half to one-quarter that of the standing tree tool. Furthermore, the trees must be cut down in the case of the log tool, which may not be possible or desirable.

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An Optimised Protocol for Fluorescent-dUTP Based SSR Genotyping and its Application to Genetic Mapping in *Eucalyptus*

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Abstract

Integration of fluorescent-dUTP in polymerase chain reaction (PCR) appears to be a sound method for fluorescence labelling of amplicons in genotyping with simple sequence repeats (SSRs) using an automated sequence

analyser. However, the method has not been explored in terms of performance optimisation and cost control. In this paper, we optimised the protocol for fluorescent-dUTP based SSR genotyping in a case study with *Eucalyptus*. A combination of low dNTP concentration (25 µM each) in PCR reaction and a touchdown PCR programme contributed to increase dramatically the fluorescent intensity of SSR amplicons, thereby facilitating

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