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GENETIC VARIATION IN ROOTABILITY OF CUTTINGS FROM ONE-YEAR-OLD WESTERN HEMLOCK SEEDLINGS

by

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ABSTRACT

One-year-old western hemlock *(Tsuga heterophylla* (Raf.) Sarg.) seedlings from three open-pollination families from eight locations in the Washington and Oregon Coast Ranges were cultured under accelerated growth conditions in a glasshouse. Forty cuttings from each of five seedlings (open-pollination siblings) per family were then placed in a rooting box in a randomized block design with five replications. Percent of cuttings rooted after one year in the bed was analyzed in a hierarchal design.

Average rooting percentage was 72.4 percent. Significant effects were associated only with replication, siblings-in-families-in-provenances (S/F/P) and with the interaction, S/F/P x replication. The last two together accounted for 73 percent of the variance.

The results indicated that additive genetic effects were not important to rooting success, but that dominance and unique clone-effects probably were.

KEYWORDS: Heritability, provenance (-genetic traits, rooting ability, western hemlock, *Tsuga heterophylla.*

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INTRODUCTION

Numerous studies have shown large genetic variation in the rootability of cuttings taken from different individuals of the same tree species. This variation has been found in western hemlock *(Tsuga heterophylla* (Raf.) Sarg.) (Brix and Barker 1975) and in other species (Mergen 1962, Ooyama and Toyoshima 1965, Wilcox and Farmer 1968, Rauter 1971, Kleinschmit 1972, Kiang and Garrett 1974, Wilcox and others 1976).

Sometimes, significant stand or population-sample effects have also been indicated. Ooyama and Toyoshima (1965) reported large differences in rooting among races in *Pinus densiflora* and in *P. thunbergii.* Their results also indicated that the racial effect varied with years. Wilcox and Farmer (1968) observed that clonal variation in rooting of *Populus deltoides* Bartr. ex Marsh was strongly related to heritable differences in budflush date, which suggests geographic variation in rootability. Rauter (1971) found that the rooting success of cuttings taken from seedlings of 18 populations of *Picea mariana* (Mill.) B.S.P. ranged from 12 to 62 percent.

Conversely, Kiang and Garrett (1974) reported that seed source of the ortet was not an important factor in rooting response in *Pinus strobus* L. cuttings taken from a provenance planting; Brix and Barker (1975) did not find a significant stand effect on the rooting of western hemlock cuttings taken from 31 plus-tree stands, 42 years of age or older, in British Columbia.

We evaluated the genetic effects associated with three levels of genetic sampling: provenance, family-in-provenance and sibling-in-family. Particularly, we were interested in the possibility of provenance variation and whether it could be related to environment at the source. One-year-old seedlings, cultured under conditions for accelerated growth in a glasshouse, were the source of the cuttings.

MATERIALS AND METHODS

Open-pollination seeds were collected from three mature trees at each of eight locations in the Washington and Oregon Coast Ranges in 1975. These locations ranged from 44°00' to 48°08' N. latitude, from 20 to 126 km from the ocean, and from 30 to 640 m in elevation. In February 1976, pregerminated seeds from each of the 24 mature trees were sown, 3 seeds per pot, into gallon containers arranged in 10 randomized complete replications on a single bench in a glasshouse. Seedlings were watered with an overhead mist spray, fertilized regularly with a balanced complete nutrient solution, and provided with supplemental light. In early summer, pots with more than one surviving seedling were thinned to one per pot. Conditions for vigorous growth were maintained until late autumn, when day length and moisture supply were reduced to induce bud set.

In February 1977, the five most uniform replications, which were the center replications on the bench, were used as a source of twig cuttings. Seedlings were 40-50 cm tall (average), well branched, and dormant. Forty cuttings, taken from each seedling (sibling), were subdivided into five

groups of eight and placed in eight-ramet rows in five randomized replications in a plastic- and shadecloth-covered rooting bed. Cuttings were 4-8 cm long and taken from all branch orders. Use of the shorter material was necessary to get the 40 cuttings.

The rooting medium was two parts peat and one part sand by volume. Cuttings and bed were kept moist with an automated overhead mist system activated by evaporation of moisture from a wire grid. Fungicide and liquid fertilizer were applied weekly. Soil temperature was maintained at approximately 21°C with subsoil heating cables. Air temperature was controlled by shadecloth and by raising the plastic sides of the frame over the rooting bed to increase ventilation. In general, air temperature around the cuttings followed the outside air temperature.

In January 1978, the number of cuttings with roots in each eight-ramet plot was determined. Although the quantity and vigor of roots varied, we tallied only the presence or absence of living roots.

Percentage of cuttings rooted was calculated for each plot, substituting 1/4n if the percentage was zero and 100-(1/4n) if the percentage was 100, where n = 8, the number of ramets per plot initially (Bartlett 1947). Percentages were transformed into arc sins in radians and analyzed according to ANOVA in table 1. Replications were considered to be random. Within-plot variance was estimated by 0.25/n (Steel and Torrie 1960, p. 158). Thirteen plots were inadvertently left out of the rooting bed and degrees of freedom for total and error (c) accordingly reduced. Because the genetic effects were random, approximate F-tests were made according to the method of Cochran (1951).

Additive genetic variance among families from open-pollination seeds is biased from natural inbreeding and an unknown proportion of full-sibs (Namkoong 1966, Squillace 1974). Based on the relationships given in Squillace (1974), we have assumed that the component of variance for families-in-provenances ($\sigma^2_{F/P}$ in table 1) estimates 1/3 of the additive variance. It may also include some of the dominance variance, but we have assumed that it did not. Heritabilities, narrow and broad sense, were estimated by the relationships

$$h^2_{ns} = \frac{\sigma^2_A}{\sigma^2_{F/P} + \sigma^2_{R(F/P)} + \sigma^2_{S/F/P} + \sigma^2_{R(S/F/P)} + \sigma^2_W} \quad \text{and}$$

$$h^2_{bs} = \frac{\sigma^2_{F/P} + \sigma^2_{S/F/P}}{\sigma^2_{F/P} + \sigma^2_{R(F/P)} + \sigma^2_{S/F/P} + \sigma^2_{R(S/F/P)} + \sigma^2_W},$$

where

$\sigma^2_{S/F/P}$ estimates 2/3 σ^2_A σ^2_D, most genetic interaction variances (Bohren and others 1965), and some c-effects,

σ^2_F estimates 1/3 σ^2_A,

(σ^2_R/P) estimates plot effects,

$(\sigma^2_R/F/P)$ estimates interaction variance of siblings-iln-fami1ies-in-provenances with replication, and

σ^2_W estimates the within-plot variance (Steel and Torrie 1960, p. 158).

Nonadditive genetic variance was calculated based on the assumption that the variance among siblings-in-families-in-provenances was made up of additive genetic variance, nonadditive genetic variance, and the interaction variance of siblings-in-families-in-provenances with replication (Namkoong and others 1966). Assuming no epistasis, nonadditive genetic variance in this example would include dominance variance (Namkoong and others 1966) plus c-effects associated with:

a) each seedling being cultured in its own unique pot microenvironment, and
b) differences in the branching habit of the seedlings, particularly as it affects twig lengths and frequencies of branches of different order.

The effect associated with item (a) would be small because of the relative **uniformity of the environmental regime in which the seedlings were cultured.**

Because dominance effects could not be separated from effects of unique seedling-microenvironment collaboration and branch morphology, we had to treat all of this variance estimate as nonadditive genetic variance.

Table I–Analysis of variance showing expected and observed mean squares for rootability of cuttings. (See text for further explanation)

Sources of variation	d.f.	M.S.	Estimated mean squares[1/]	EMS code	F	F[1/2/]
Replication (R)	4	.2770	$\sigma^2 R(S/F/P) + S\sigma^2 R(F/P.) + sf\sigma^2 RP + sf P\sigma^2 R$	E_1	3.91*	
Provenance (P)	7	.5184	$\sigma^2 RiS/F/P) + r\sigma^2 (S/F/P) + s\sigma^2 R(F/P) + sr\sigma^2 F/P$ + $sf\sigma 2_{Rp}$ + $Sfr\sigma 2_{p}$	E_2		1.78[3/] n.s.
Error a (R x P)	28	.0709	$\sigma^2 R(S/F/P) + S\Sigma\tau^2 R(F/P) + sf\sigma^2 RP$	E_3		
Families/P	16	.2516	$\sigma^2 R.(S/F/P) + r\sigma^2 (S/F/P) + S\sigma^2 R(F/P) + sr\sigma^2 F/P$	E_4		1.18[4/] n.s.
Error b (R x F/P)	64	.0582	$\sigma^2 R(S/F/P) + S\sigma^2 R(F/P)$	E_5		
Siblings/F/P	96	.2181	$\sigma^2 R(S/F/P) + r\sigma^2 (S/F/P)$	E_6	2.98**	
Error c (R x S/F/P)	371	.0733	$\sigma^2 R (S/F/P)$	E_7	2.35**	
Within plot	4109	.0312	$\sigma^2_w - 0.25/n$			

1/ σ^2_R = Variance component for replications;

σ^2_P = Variance component for provenances;

$\sigma^2_{F/P}$ = Variance component for families-in-provenances;

$\sigma^2_{S/F/P}$ = Variance component for siblings in families-in-provenances;

n = Number of cuttings per plot = 8;

r = Number of replicates = 5;

p = Number of provenances = 8;

f = Number of families/provenance = 3;

s = Number of siblings/family/provenance = 5.

2/ Approximate F-test.

3/ $F' = (E_2 + E_5)/(E_3 + E_4)$

d.f. (numerator) $= (E_2 + E_5)^2/\left(\frac{E_2^2}{f_2} + \frac{E_5^2}{f_5}\right) = 8.6$

d.f. (denominator $= (E_3 + E_4)^2/\left(\frac{E_3^2}{f_3} + \frac{E_4^2}{f_4}\right) = 25.2$

4/ $F' = (E_4 + E_7)/(E_5 + E_6)$

d.f. (numerator) $= (E_4 + E_7)^2/\left(\frac{E_4^2}{f_4} + \frac{E_7^2}{f_7}\right) = 26.6$

d.f. (denominator) $= (E_5 + E_6)^2/\left(\frac{E_5^2}{f_5} + \frac{E_6^2}{f_6}\right) = 139.2$

5/ Probability of a larger F: n.s. > .05, * = .05, ** = .01.

RESULTS AND DISCUSSION

Rooting percentages (retransformed values) for the clones ranged from 33.2 to 96.9 and averaged 72.4 percent. Average rooting of provenances ranged from 65.2 to 81.9 percent. The range of average rooting percentages for families-in-provenances was less (66.5 to 77.8) and for siblings-in-families-in-provenances greater (52.5 to 84.9) than the range for provenances.

Components of variance are given in table 2. Neither provenance nor family-in-provenance variances were significant; siblings-in-family-in-provenance variance was highly significant. Coefficient of variation associated with error c (table 1) was high, 33 percent, even though the test included 40 cuttings per clone, which illustrates the difficulty of getting precision in a test of this type.

Additive genetic variance was estimated to be 0.0057, giving a narrow-sense heritability of 0.04 and a broad-sense heritability of 0.23. Non-additive genetic variance was estimated to be 0.0252, about four times the additive variance.

Given the sampling stratification we used, significant provenance-variance would have suggested an association between rootability and some geographically varying genetic factor, for example, phenological state of the plant. An excess of family-in-provenance variance over sibling-in-family variance would have indicated an additive heritable control of rootability. This test did not provide evidence that either of these factors was important in the rooting of cuttings taken during the dormant season from 1-year-old western hemlock.

Under near-optimal cultural conditions, 90+ percent rooting of cuttings from hemlock seedlings can be expected (Brix 1978, and Stephen Ross, Forest Research Center, Weyerhaeuser Company, Centralia, Washington, personal communication). Our conditions were less than optimal, probably because of lack of air temperature control over the rooting beds. At about 70 percent rooting success, comparatively large effects were associated with siblings-in-families and with interactions between siblings and replication. Statistically, these effects showed up as nonadditive genetic variance.

Variance from dominance alone is not normally expected to exceed additive genetic variance (Comstock and Robinson 1948), although it can for highly sensitive traits (Gene Namkoong, USDA Forest Service, Genetics Department, North Carolina State University, Raleigh, personal communication). In our test, nonadditive variance was about four times additive variance. Because growing conditions within the glasshouse were relatively uniform, the siblings-in-families-in-provenance variance probably reflects genetic differences to a much greater extent than it does environmental precondi-tioning. However, the genetic differences may also include clone- or c-effect associated with crown form, branching habit, and twig availability of the individual seedlings. Crown form and branching habit are under genetic control, but their effect would vary depending on the number of cuttings needed and the relative size of the seedling. If the crown characteristics of the seedling are included, most of the genetic variation in rootability of cuttings from these dormant hemlock seedlings was associated with the individual plants, and very little with the families and the provenances.

Table 2--Estimated components of variance for the three levels of sampling and their interaction with replication

Component	Estimate	Percent
Replication (σ^2R)	.0017	1
Provenance (σ^2_p)	.0034	2
Replication x Provenance (σ^2_{Rp})	.0008	1
Family/P ($\sigma^2_{F/p}$)	.0019	1
Replication x Family/P ($\sigma^2R(F/P)$)	0	0
Sibling/F/P ($\sigma^2_{s/p/p}$)	.0290	21
Replication x sibling/F/P ($\sigma^2_{R(S/F/p)}$)	.0733	52
Within plot (.25/n) (σ^2_W)	.0312	22
	.1413	100

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