Multiple Propagation Effects on Genetic Estimates of Rooting for Western Hemlock

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Abstract. Analysis of clonal variation for two rooting traits of western hemlock [Tsuga heterophylla (Raf.) Sarg.] clones indicated that realized genetic gain would be improved by more effective partitioning and reduction of environmental differences associated with clones. Interactions between clones and multiple propagation dates were significant, but clone rank changes between dates were minor. Number of rooted cuttings per plot (RC) and number of main roots per cutting (MR) were more highly correlated genetically (1.06) than phenotypically (0.36). Broad-sense heritabilities (heritabilities = 0.62 and = 0.79 for RC and MR, respectively) and predicted genetic gain from clonal selection were moderately high. Both the percentage of rooted cuttings and root system quality could be rapidly improved by the clonal selection procedures used.

Compared with seed propagation, asexual propagation of “select” trees offers gains in growth, pest resistance, uniformity, and other desirable traits (Roulund, 1973). A vegetative propagule (ramet) is a genetic copy of the ortet (original seedling); thus, desirable traits can be transmitted to ramets to produce a clone. Vegetative propagules with superior traits can be used for genetic studies, to establish seed orchards, or be released as named cultivars.

Variation observed among ramets within a clone (infraclonal) originates from changes in physiological factors due to environmental effects. Differences in propagule response to environmental factors such as light, moisture, fertility, and temperature can determine how the genetic potential of a clone is expressed. Environmental factors acting on the ortet may be carried forward in propagules of a clone for various periods (Cannell et al., 1978; Libby and Jund, 1962). Environmental factors that persist to affect successive generations and that inflate differences among clones are generally referred to as C effects (Wilcox and Farmer, 1968).

Estimates of total genetic variation can be inflated if substantial environmental variation is confounded with infraclonal variation. Biased estimates of genetic variation influence estimates of genetic parameters such as heritability, correlations among traits, and expected gain from selection. Traits measured shortly after cloning are most subject to overestimation of genetic variance due to C effects (Libby and Jund, 1962). The presence and size of environmental effects may have an important bearing on the success of a tree improvement program since they might cause the erroneous selection of genetically inferior clones.

Various C effects that influence rooting traits of trees have been observed. As the age of the ortet increases, the rooting ability of cuttings generally decreases (Brix and van den Driessche, 1977). Tree species vary in the rate of ontogenetic decrease (Foster et al., 1981; McAlpine and Jackson, 1959; Roulund, 1973), with the loss of rooting ability being generally irreversible (Olesen, 1978). Roulund (1973) found an average increase in rooting of 2.5% per whorl as cutting origin changed from the top to the bottom of the crown in Norway spruce [Picea abies (L.) Karst.]. Plant health and vigor have been observed to have a very positive effect on rooting ability (Dormling and Kellermam, 1981). Other factors affecting rooting ability include reproductive organs on cuttings (Burden and Shelbourne, 1974), position within a branch, and cutting size (Ying and Bagley, 1977).

In cottonwood (Populus deltoides Bartr.), C effects accounted for 9% to 11% of the total phenotypic variation (one-third the total genetic variation) for two rooting traits (Wilcox and Farmer, 1968). Analysis of phenotypic variation for five rooting traits of western hemlock demonstrated that the potential bias to genotypic values of clones owing to C effects was significant, but heritability and gain estimates were only slightly biased (Foster et al., 1984). These authors suggested that efforts be made to minimize or control variation owing to C effects when selecting clones for higher rooting potential.

Clonal variation in rooting western hemlock (hereafter referred to as hemlock) has been reported by several researchers. Rooting studies conducted on a juvenile population (6 to 20 years old) and a mature population (19 to 60 years old) of hemlock found that broad-sense heritabilities for proportion of rooted cuttings per ortet were 0.53 in juvenile and 0.55 in mature populations, while for number of main roots per rooted cutting, heritabilities were 0.31 in juvenile and 0.27 in mature populations (Foster et al., 1981). Product–moment correlations of the two traits with age of ortet, calculated separately for the two populations, were significant. They ranged between –0.14 to –0.21 and indicated that a small decline in rooting ability occurs as ortet age increases. When open-pollinated families of 1-year-old hemlock seedlings were used as ortets, broad-sense heritability was estimated on an individual plant basis as 0.23 and narrow-sense heritability as 0.04 for the percentage of rooted cuttings per ortet (Sorensen and Campbell, 1980).

This study had three objectives: 1) to evaluate the importance of several environments on the estimation of genetic control of two rooting ability traits among clones; 2) to determine the
to predict the level of genetic gain from selection of the best clones in the population.

Materials and Methods

Twenty hemlock ortets were randomly chosen from trees included in a low-intensity, comparison-tree selection program located in Crown Zellerbach Corp.-managed forests between Clallam Bay, Wash. (48°15’N latitude) and Tillamook, Ore. (45°30’N latitude) in the Coast Ranges between 0 and 300 m elevation. Ortets ranged between 28 to 62 years, estimated at breast height. The 20 ortets (clones) were assumed to be a random sample from the wild population, especially for rooting ability traits, since trees were not chosen for rooting characteristics, and analysis of 5-yr heights from progeny tests of trees in the program (Foster and Lester, 1983) indicated no significant differences in total height from random seed collections.

Stem cuttings were collected from the previous year’s last growth cycle in the top one-third of the crown of parent trees in Jan. 1976 and were rooted in mist benches. The rooted cuttings of each ortet were potted in Feb. 1977. The cuttings (primary ramets) were grown in a greenhouse for two growing seasons and uniformly hedged in the late winters of 1978 and 1979 to develop stock plants (shorter than 0.5 m). All ramets were grown in 11.4-liter pots with the same potting medium (1 sand : 1 peat : 1 perlite, by volume) and was steam-sterilized before use.

The rooting chamber consisted of a wood-framed, vinyl tent measuring 16.9 × 1.2 × 0.8 m (length/width/height) and was raised above 15°C. The humidity was monitored and the mist system was thermostatically controlled system to maintain the temperature below 21°C and the relative humidity above 80%.

Degree of genetic, phenotypic, and environmental relationship between the two rooting traits: and 3) to predict the level of genetic gain from selection of the best clones in the population.


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Table 1. Analysis of variance for number of rooted cuttings per plot (RC) and main roots per rooted cutting (MR) of hemlock.

| Source of variation | df | RC | MR | Expected mean squares |
|---------------------|----|----|----|-----------------------|
| Dates(D)            | 1  | 27.66** | 145.20** | σ^2 + bσ_{DC} + σ_{BD} + ceσ^2 |
| Blocks(B)/D         | 14 | 1.02ns | 2.28ns | σ^2 + ceσ^2 |
| Clones(C)           | 19 | 6.47** | 19.88** | σ^2 + bσ_{DC} + bσ_{BD} |
| D × C               | 19 | 2.42** | 4.45** | σ^2 + bσ_{DC} |
| Error               | 210 | 1.22 | 1.83 | σ^2 |

where d, b, and c are values for the number of dates(2), blocks(8), and clones(20), respectively; σ^2_d is variance among dates; σ^2_{BD}, is variance among blocks within dates; σ^2_c is variance among clones; σ^2_{DC}, is variance owing to interactions of dates and clones; σ^2 is the error variance.

A synthetic F test, after the technique of Cochran (1951) was used to test for differences among dates.

**Nonsignificant at P = 0.05 or significant at P = 0.05 or 0.01, respectively.

Table 2. Estimated variance components and se^2(in parentheses) for number of rooted hemlock cuttings per five cutting plot (RC) and number of main roots per rooted cutting (MR).

| Variance component | Estimate | Total variation (%) | Estimate | Total variation (%) |
|--------------------|----------|---------------------|----------|---------------------|
| σ^2_d              | 0.16 (0.141) | 9                   | 0.88 (0.741) | 22                  |
| σ^2_{BD}           | 0.00 (0.019)  | 0                   | 0.02 (0.041) | 1                   |
| σ^2_c              | 0.25 (0.133)  | 14                  | 0.96 (0.154) | 24                  |
| σ^2_{DC}           | 0.15 (0.095)  | 8                   | 0.33 (0.173) | 8                   |
| σ^2                | 1.22 (0.118)  | 69                  | 1.83 (0.179) | 45                  |

Namkoong (1979).

Calculated from observed mean squares in Table 1.

Results and Discussion

The results of this study allow the partitioning of phenotypic variation into total genetic variation and environmental effects. Estimates of genetic variation, heritabilities, and predicted genetic gain from clonal selection were influenced by environmental effects. Significant variation was indicated among the 20 clones for both rooting traits (Table 1). Clonal variation with respect to RC and MR accounted for 14% and 24% of each trait’s total model variation, respectively (Table 2). Variation among blocks per date was nonsignificant for both traits (Table 1). This result indicates a high degree of uniformity existed within the propagation environment during both rooting trials. Based on overall average rooting of 59% for Dec. 1980 and 40% for Feb. 1982, propagation date constituted a significant source of total variation for both MR (22% of total variation) and RC (9% of total variation) (Tables 1 and 2).

Date × clone interactions were significant for both traits (Table 1), indicating that average performance by clone may vary between setting date. For both MR and RC, the interaction accounted for 8% of total variation (Table 2), which is =40% the size of clonal variation. A Spearman rank correlation calculated between the clone MR mean rankings for the two dates indicated a significant (P < 0.05) correlation (r = 0.81) in rank; hence, only minor rank changes among clones had occurred between the two dates. The Spearman rank correlation (r = 0.43) between the RC clone mean rankings for each date barely failed (P = 0.06) to meet the standard 5% significance level. One clone, ranked lowest for the first propagation date and fifth highest for the second date, accounted for one-third of the variation in rank change for RC. With this one exception, only minor rank changes were indicated for RC for the two dates. Therefore, although the ANOVA indicated significant shifts in clonal rooting between setting dates, rank correlation was high enough to indicate a rather similar rooting performance for clones between setting dates.

Broad-sense heritabilities on a clone–mean basis were 0.62 for RC and 0.79 for MR (Table 3), indicating observed differences in rooting ability among clones in this experiment were due largely to genetic effects. If the variation due to propagation date had been completely confounded with genetic variation by making estimates based on a single propagation date, heritabilities would have been inflated by the clone × date interaction. This misguided result would have led geneticists to think that rooting ability was more highly inherited than it is.

Estimates for heritabilities of hemlock rooting traits on a clone–mean basis by Foster et al. (1984) were 0.87 for RC and 0.92 for MR, which were in agreement with data recalculated on a

Table 3. Estimated broad-sense heritabilities and potential genetic gains based on clonal selection for number of hemlock rooted cuttings per five cutting plot (RC) and number of main roots per cutting (MR).

| Mean (X) | Phenotypic SD (σX) | Range of clone means | CV (%) | Heritability |
|----------|-------------------|----------------------|--------|-------------|
| 3.0      | 1.3               | 1–5                  | 43     | 0.62        |

Genetic gain^a

| Measured units | Percentage | Genetic gain |
|----------------|------------|--------------|
| 0.73           | 25         | 1.10         |

^aAssuming a 10% selection intensity, i = 1.755.
clone–mean basis from an earlier hemlock study (Foster et al., 1981). These reported estimates are higher than estimates of this study (RC = 0.62 and MR = 0.79). Differences between estimates of heritability in this study and the earlier estimates may be due to the previous studies having been based on only one propagation date.

In study of loblolly pine (Pinus taeda L.), Foster (1990) found significant differences in rooting percentages among three propagation dates. Additionally, he partitioned total genetic variation into additive, dominance, and epistatic effects. He found virtually all of the genetic variation was due to additive genetic control. Data from the present study indicate that one propagation date would bias estimates of heritability with an unpartitioned $\sigma^2_G$ confounded with $\sigma^2_e$ in the numerator value used to calculate heritabilities. Estimates based on one date are biased upward ($\sigma^2_C + \sigma^2_{DC}$) by 30% for RC and 16% for MR.

Predicted genetic gains by selecting the top 10% of the clones (two of 20) expressed as a percentage improvement over the population mean were 25% for RC and 30% for MR (Table 3). This level of selection intensity was chosen to be representative of a reasonable level for a normal-sized program that could contain several hundred clones. Variation among clones for both rooting traits was large, contributing to the substantial genetic gain predicted for each trait. Considerable differences in clone means occurred; the phenotypic cv was $=45\%$ for both traits (Table 3). The large genetic gains (Table 3) seem reasonable because genetic gain depends heavily on both heritability and variation among clone means. This relationship suggests that one generation of clonal selection for rooting traits could produce substantial changes in population means.

Our results indicated that RC and MR are closely associated genetically and phenotypically. The genetic correlation was 1.06 (representing perfect correlation; that is, the same genes condition both traits), the phenotypic correlation was 0.36 and the environmental correlation was 0.17. Since the genetic correlation is high and positive, selection for either rooting trait based on clone-mean performance would result in a shift of the other character in the same direction. Direct selection for MR has a higher heritability and would produce a higher correlated response in RC, whereas RC can be evaluated faster and still produce good response for both traits. After one generation of selection among hemlock clones, improvement in the proportion of rooted cuttings and quality of the root system is predicted to be substantial.

Implications

This study employed four techniques that have been proposed (Foster et al., 1984) to increase precision in selecting genetically superior clones for rooting characters. The first technique involved the collection of cuttings from the same crown position in each ortet selected for cloning. This reduced the C effects associated with rooting ability that have been demonstrated to occur as cutting position changes within the crown of an ortet (Foster et al., 1984; Roulland, 1973).

The second technique involved secondary cloning, the process of rooting the cuttings (primary ramets) from the ortet, then taking cuttings (secondary ramets) from the rooted primary ramets. Uniform treatment of the primary ramets increased the vigor and uniformity of cuttings used in this study to produce secondary ramets of each clone. Secondary cloning served to reduce physiological differences associated with the original ortet (i.e., nutrient levels) that could bias a clone’s ability to root (Preston et al., 1953).

The third technique involved the randomization of cuttings within the rooting chamber at multiple dates. Heritability and phenotypic $\sigma^2$ are affected by blocking and propagation under multiple environments (dates) that indirectly influence expected genetic gain. Conducting the rooting trials on multiple dates allowed a better assessment of the clonal rooting performance as compared with a single date. The goal of our program is to select clones that root well repeatedly. In an analytical sense, the genetic variance (estimated by $\sigma^2_G$) is no longer confounded with the genotype × environment interaction (estimated by $\sigma^2_{GE}$) when the rooting ability of the clones is assessed in two or more trials. Furthermore, the multiple trial (date) experiment provides a more realistic estimate of the phenotypic $\sigma^2$ deviation (since $\sigma^2_B$ is removed) and of the heritability estimate (since $\sigma^2_C$ and $\sigma^2_{GC}$ are not confounded).

The fourth technique was to select clones for rooting traits with high heritability. MR and RC have previously been demonstrated to have high heritability for hemlock clones selected from the wild population (Foster et al., 1984). Genetically superior individuals are easier to identify if heritabilities are high, because the phenotype more closely reflects the genotype and selection is more efficient.

Using these horticultural techniques to increase precision in clonal selection increases propagation cost. Secondary cloning, for example, requires material to be maintained for several years and lengthens the evaluation process. This would limit the number of clones that could be evaluated with given resources and reduces predicted gain per unit time. The projected increase in realized gain by removing environmental factors confounded in genetic estimates of rooting ability may offset these costs because superior clones can be identified more precisely.

Potentially, the percentage of rooted cuttings and root system quality could be rapidly improved through clonal selection in hemlock. Increased rooting ability would have a large economic impact on production cost by reducing the size of cutting orchards, rooting facilities, labor requirements, and aftercare facilities for a given production volume. Improved root system quality may also speed outplanting to the field and enhance subsequent field growth.

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