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Using clones to regenerate a species new to clonal reforestation presents the forest manager with many problems. A number of interrelated and interdependent research and development activites are needed to answer these technical questions. Network diagraming was used for scheduling research activities and for indicating interdependencies among activities. The resultant diagram, although developed specifically for western hemlock [Tsuga heterophylla (Raf.) Sarg.], represents a program which may be useful for other species in which clonal reforestation is considered to be potentially appropriate.

Once the network diagram was completed, several activities were examined in detail by experiments. The first group of activities dealt specifically with clonal variation (and its components) for five rooting traits and demonstrated that clonal variation was due to both genetic and C effects (persistent environmental effects). The potential bias to genotypic values of clones due to C effects is significant, but heritability and gain estimates are only slightly biased.

The five rooting traits were highly heritable (H = 0.87 to 0.92), and predicted genetic gain from clonal selection was substantial. Genetic correlations between pairs of traits were generally high (0.66 to 0.99); therefore, when selecting for any one trait, correlated responses can be expected in other traits.

The second group of activities examined components of clonal variation for juvenile height (HT) as well as associations between rooting traits and subsequent height growth of rooted cuttings. As with the rooting traits, C effects in HT were a significant proportion of the total genetic variation. HT was found to be under strong genetic control (H = 0.81), and genetic correlations between HT and rooting traits ranged from 0.37 to 0.59. A selection index containing both juvenile height, HT, and a rooting trait, VOL, would result in the application of selection pressure to both traits simultaneously.

Genetic Considerations in Cloning Western Hemlock

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GENETIC CONSIDERATIONS IN CLONING WESTERN HEMLOCK

CHAPTER I. INTRODUCTION

Reforestation using clones of western hemlock [Tsuga heterophylla (Raf.) Sarg.] has several potential advantages over traditional reforestation with seedlings. The main advantage arises from the genetic gains achieved through clonal selection. Variation among clones can be expressed as:

$$V_C = V_G + V_E$$

where, V_C is clonal variation, V_G is total genetic variation, and V_E is environmental variation. An additional component due to genotype x environment interaction will be ignored for simplicity. Total genetic variation can be further subdivided into its components (Becker, 1975):

$$V_G = V_A + V_D + V_{AA} + V_{AD} + V_{DD} + \cdots$$

where V_A is additive genetic variation, V_D is dominance genetic variation, V_{AA} is additive by additive epistatic variation, etc. Since the components are difficult to partition, geneticists often combine dominance and epistatic genetic variation into the general category of non-additive (V_{NA}) genetic variation. Genetic gain is achieved by selecting among clones within a population and reforesting with selected clones, thereby utilizing the total genetic variation, V_G, present in the population.

Most tree-breeding programs rely on sexual propagation, and genetic gain results from using additive genetic variation (V_A).

If a trait in a population of trees has a significant amount of non-additive genetic variation, genetic gain from a clonal program will always exceed the gain from an improvement program where propagules for reforestation are produced sexually. Unlike clonal (asexual) propagation, sexual propagation involves genetic recombination among genes of the selected parents. Genetic recombination may break up specific interactions between alleles at each locus (dominance) and interactions between alleles at different loci (epistasis). This mixing of alleles negates the use of dominance (V_D) and epistatic (V_{AD}, V_{DD}, etc.) genetic variation in most improvement programs based on selection and breeding.

The difference in the variation that can be used by the two approaches arises from the manner in which propagules for reforestation are produced. In both approaches, superior individuals in the population are identified. In a sexual program, the selected parents are then mated to produce an offspring population; in an asexual program selected parents are clonally propagated to produce an offspring population, thereby avoiding sexual recombination.

The amount of additional gain achieved through clonal selection depends upon the magnitude of the non-additive genetic variation. Genetic tests demonstrate that the non-additive component can make up a large portion of genetic variation for traits in populations of trees. Genetic variation in fifth-year height in one population of loblolly pine (Pinus taeda L.) included half as much dominance variation as additive genetic variation (Stonecypher et al., 1973). Dominance variance exceeded additive variance for individual-tree

volume in the same study. For rooting traits of stem cuttings, Foster (1978) and Sorensen and Campbell (1980) found that non-additive genetic variation was larger than additive variation. Clonal selection may therefore offer larger genetic gains than the more traditional tree improvement programs.

Even with the expectation of larger genetic gains through clonal selection, some problems exist. Aging of clones can sometimes reduce rooting ability and growth rate (Libby and Hood, 1976). Abnormal growth habit (plagiotropism) may cause the trees to grow poorly and have crooked stems (Olesen, 1978). Non-genetic, yet persistent, factors termed C effects (e.g., poor growth in rooted cuttings reflecting low vigor in the parent) which are associated with certain clones can also influence propagule growth (Wilcox and Farmer, 1968). C effects are an especially troublesome type of environmental effect (V_E) whose impact on clonal selection and growth must be determined before proceeding with commercial clonal reforestation.

This thesis is divided into four chapters including the Introduction. The second chapter presents an analysis of research and development activities needed to evaluate clonal reforestation with rooted cuttings. To be successful, a clonal reforestation program requires information from preliminary research and development studies. In order for these studies to proceed in the most efficient manner, an underlying framework to order them should be developed. This framework should not only indicate the sequence of necessary research and development activities but should also estimate the duration of each activity and interdependencies among them. Information

from the activities should enable the manager of the reforestation program to make appropriate and timely decisions. In addition, a framework for the activities should minimize losses of time and money that may be expected if the incorrect species is chosen for reforestation. A network diagraming technique was employed in this study to order the numerous activities. A discussion of the use of the diagram concludes the chapter.

Several of the activities from the network diagram are then investigated at length. The activities were chosen because of their importance to a rooted cutting program. Although the network diagram applies to any tree species, results of experiments in the activities studied apply only to western hemlock. These include studies of genetic and non-genetic factors affecting the rooting of cuttings and the subsequent height growth of the propagules. Variation in the rooting ability of hemlock cuttings is reported in the third chapter. Clonal reforestation of hemlock in the near future will likely use rooted cuttings because hemlock cuttings root with relative ease, even when the ortet (donor plant) is mature (Brix and Barker, 1975). Once the cuttings do root, however, variation is likely to exist in the quality of root systems, which subsequently may have a large effect on early growth of the rooted cuttings. Hypothetically, an adequate root system should allow the rooted cutting to express its genetic potential for subsequent growth; conversely, a poor quality root system might limit propagule growth. The magnitudes of genetic variation and C effects variation are estimated and compared for several traits related to rooting ability and root systems of rooted cuttings. In

addition, heritabilities and expected gains due to clonal selection are calculated. Genetic correlations between various rooting traits are estimated and used to calculate correlated responses when selection is applied to a single trait.

The relationship between rooting ability and subsequent height growth of rooted cuttings is examined in the last chapter. Previous evidence suggests that both rooting ability (Sorensen and Campbell, 1980; Foster, Martin, and Caldwell, 1981) and early height growth (Foster and Lester, 1983) in western hemlock may be under strong genetic control. Thus, both are amenable to improvement through clonal selection. Furthermore, if these two traits are positively correlated, clonal selection may be effective in improving both traits simultaneously. In Chapter IV, first-year heights of hemlock rooted cuttings are related to measures of the root system through correlation analyses. A selection index is then developed which could be used to improve both rooting ability and height growth.

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CHAPTER II. NETWORK ANALYSIS OF RESEARCH AND DEVELOPMENT TO EVALUATE A REFORESTATION PROGRAM UTILIZING ROOTED CUTTINGS

Introduction

Foresters currently use rooted cuttings for reforestation in several parts of the world, but clonal reforestation still represents a new approach to regeneration for many tree species. Theoretically, clones offer many advantages for using genetic variation to increase productivity. Unfortunately, reliable quantitative estimates of these advantages generally do not exist. Also, potential increases in productivity may be offset by costs associated with the rooting process or losses due to non-genetic artifacts of propagation such as immediate or delayed physiological deterioration of growth due to aging of clones and malformation of tree stems due to plagiotropism. Using clones, therefore, raises many questions. The answers to these questions are often specific to a species or management goal. Furthermore, the design of experiments sometimes depends on results from earlier experiments. Negative economic, biological, or genetic results of experiments may even preclude the use of clones of some species altogether.

There has recently been a surge in research and development to answer questions about rooting and growing cuttings of forest trees. The scope of the experiments has often been necessarily narrow; consequently, they often provide seemingly isolated bits of information. A conceptual basis for structuring research questions and the means for getting answers seems to be lacking. Obviously, the efficient

management of research and development to evaluate the role of rooted cuttings in reforestation presupposes an orderly framework.

In this chapter, a framework for guiding research and development to evaluate reforestation by stem cuttings is developed through network analysis, a management technique useful for structuring any problem or project which involves several interrelated and time-dependent activities. Within the framework, research and related activities are enumerated, interrelations between activities are presented, and a scheduled sequence of activities is suggested. Furthermore by examining an array of activities and their time dependencies, previously unrecognized problems come to light and can be considered in decision making.

Theoretically, following the suggested sequence of activities reduces the risks inherent in starting clonal reforestation because the operational program is guided by results from research and development. Risk, in this context, denotes an increased chance of program failure or losses in time or money due to incorrect decisions. If limited budgets and time frames cause some scheduled activities to be deleted in practice, it must be recognized that the subsequent loss in information will lead to increased risk.

Davis (1968) and Husch (1970) were among the first to apply network analysis in forestry. Campbell (1974) used the procedure as a descriptive aid in an evaluation of Norwegian tree improvement practices. He also considered clonal reforestation as an option in Norway but did not pursue the topic. Choice analysis, another recently developed management technique (Velk, 1978), can be used to prioritize

activities and to evaluate risk involved when making choices among activities. This has not been done here because choices depend strongly on the individual situation (i.e., species and management option).

General Network Analysis Description

The technique of network analysis varies in complexity depending on the particular problems and needs of the program. There are six basic steps, however, in any network analysis (Husch, 1970):

1. Determine specific project objectives.
2. Select project activities to satisfy the objectives and determine the time and resources required for each activity.
3. Construct a network diagram showing the sequence interdependencies and interrelationships of the activities.
4. Calculate time estimates for project completion and identify the critical path (to be defined) in the diagram.
5. Evaluate and modify the model, and schedule project activities using the revised model.
6. Use the network diagram and proposed schedule to monitor project progress.

The actual diagram is a graphical representation of the ordered sequence of steps (research and development activities) in the problem. Each activity begins and ends at a node (an event), and the direction of travel through the diagram is indicated by arrows. Each

event represents an instant in time and can be either the start or end of one or more activities.

Several rules must be followed in constructing and understanding the diagram (Husch, 1970):

"1. Each activity is represented by only one arrow bounded at each end by an event represented by a small numbered circle. The event at the tail of the arrow represents the beginning of the activity and the event at the head indicates its completion.

2. Length, curvature, or direction of arrows have no significance.

3. Several activities can begin at a single event (a burst event). Similarly several activities can terminate at an event (a merge event).

4. Before any activity can begin all preceding activities must be completed.

5. Two kinds of arrows are shown in the diagram, one having a solid line and one a broken line. The solid line indicates an activity that requires time or resources to complete. The broken line is a 'dummy' activity which uses no time or resources, but indicates a dependency relationship between events.

6. The length of the arrows is not scaled to the duration of the activity.

7. Events are numbered for identification purposes. An activity can be identified by citing the numbers of the two events bounding it, ..."

The critical path is the path (sequential order of activities between the beginning and ending nodes) through the entire diagram requiring the longest time to complete and is composed of critical activities (activities along the critical path). All other paths in the diagram are shorter. The difference in time between the critical path and any other path is called the slack time. A delay in any activity along the critical path will cause a delay in the project;

but delays in any other path, up to the slack time, will not cause a delay in the project.

Clonal Reforestation Network Analysis

Development of Network Analysis

The network analysis procedure guided the development of the network analysis for the clonal reforestation project. Initially, a project objective statement was developed. The project objective was: construct a research and development program to assess the value of a clonal reforestation project using rooted cuttings.

Once the project objective was formulated, the next level of objectives was defined. These objectives were more detailed than the project objective and directly supported it. An example of this level of objective is: Determine genetic gains achieved from selecting clones. Most of the objectives at this level were still too general to solve directly.

To reach a point where the objectives could be addressed with research and development activities, the objectives were refined further. This process resulted in a pyramid-like arrangement of objectives with the project objective at the top and working level objectives at the lowest level (Husch, 1970).

The working level objectives were stated in such a way that they could be satisfied with specific activities. An example of a working level objective is: Provide information on the degree of inheritance for growth traits. The activity which satisfies this objective is then: Determine heritabilities for growth traits.

Once activities were developed to meet all of the working level objectives, duration times were estimated for each activity (Table 1). The duration of each activity constitutes the length of time required to complete the activity. Since duration times are species dependent, durations apply only to western hemlock. Biological considerations influence most of the durations. For example, hemlock cuttings require about six months to root therefore any activity which includes the rooting of cuttings must last for a minimum of six months. Some activities, such as the calculation of genetic parameters (310;400, 310;410, 400;420, and 400;430, see Table 1), require little time once the data from the long term growth experiments (200;310) are available. The author estimated duration times (in half year intervals) based on his experience with the species.

An important objective in this analysis was to minimize the risks associated with incomplete information, for example, the information obtained from juvenile measurements (in lieu of rotation length) in yield trials. The long duration times of some activities stem directly from this objective.

Activities were then arranged in the network diagram format. The activities are interrelated, often interdependent, and some require much more time for accomplishment than others. Hence, the scheduling network is complicated (Figure 1). An activity cannot start until all information necessary for its completion is available. For example, one cannot study the physiological requirements for rooting (100;110) until a species has been chosen (80;90). The activities were scheduled by placing them as close to the beginning point as possible considering the information required for their completion. Hence, the

Table 1. Activities, identification numbers, and durations for a network analysis of reforestation research using rooted cuttings.

Activity	Identification number	Duration (years)
Study market conditions by species and product	10;20	0.5
Determine rotation length by species and product	10;30	0.5
Determine number of propagules needed per year	10;40	0.5
Determine feasibility of managing different species given land base and funding	10;50	1.0
Conduct literature review of rooting ability and rooted cutting performance by species	10;60	0.5
Decide whether a clonal reforestation program is feasible for a species	70;80	0.5
Determine species to be used in program(s)	80;90	0.5
Build research rooting facility	90;100	0.5
Study physiological requirements for rooting	100;110	2.0
Compare early growth of rooted cuttings to seedlings	110;120	5.0
Study cutting quality versus rooting ability	110;130	2.0
Conduct rooting trials varying ortet age	110;140	2.0
Determine preliminary cost of a rooted cutting and compare to a seedling	110;150	1.0
Study genetic variation in rooting traits and calculate heritability, genetic correlation, and gain	110;160	5.0
Study effect of ortet crown position on rooting and subsequent cutting growth	110;170	5.0

Table 1. (continued)

Activity	Identification number	Duration (years)
Calculate impact of C effects (topophysis and cyclophysis) on rooting traits	160;180	2.0
Decide whether a clonal reforestation program is feasible for this species	190;200	0.5
Assess morphological differences between rooted cuttings and seedlings due to ortet age effects	200;280	30.0
Study differences in growth performance of rooted cuttings versus seedlings over duration of rotation	200;290	45.0
Assess morphological differences between rooted cuttings and seedlings due to propagation technique	200;300	30.0
Evaluate cost effectiveness of a rooted cutting reforestation program as compared to a seedling reforestation program	290;570	1.0
Study relationship between rooting ability and growth of cuttings	200;220	10.0
Develop biological model which combines genotypic and environmental factors, using short term trials, to predict performance of rooted cuttings	200;230	15.0
Study genetic variation patterns and estimate genetic parameters for growth traits	200;310	45.0
Assess magnitude of genotype x environment interaction	310;400	0.5
Assess reliability of early testing information to predict mature genetic performance	310;410	2.0
Determine heritability, genetic correlation, and gain for growth traits	400;420	0.5
Assess magnitude of C effects on growth traits	400;430	0.5

Table 1. (continued)

Activity	Identification number	Duration (years)
Determine value functions for different traits	400;440	2.0
Choose traits for selection	420;520	0.5
Study multiple trait selection systems	520;540	1.0
Select most efficient multiple trait selection system	540;550	0.5
Determine potential gain from multiple trait selection	550;560	0.5
Develop hedging, serial propagation, and other cutting production systems	200;250	10.0
Determine relationship between cutting production system and C effects	250;330	30.0
Calculate cutting production rate for the different production systems	250;320	0.5
Determine percent usable cuttings produced in each system	320;390	0.5
Design cutting production system	390;450	1.0
Calculate cost of a usable cutting	450;510	0.5
Study relationship between ortet age and cutting growth	200;260	30.0
Develop curves of rooted cutting growth versus ortet age	260;340	1.0
Study rooting, handling, and delivery systems for cuttings	200;270	1.0
Study handling and delivery needs	270;370	0.5
On a production scale, determine maintenance needed during rooting	270;360	1.0
On a production scale, determine environmental conditions needed during rooting	270;350	1.0
On a production scale, determine aftercare needed following rooting	350;380	1.0

Table 1. (continued)

Activity	Identification number	Duration (years)
On a production scale, determine physiological conditioning needed prior to outplanting	380;460	2.0
Assess survival of rooted cuttings from rooting to preparation for outplanting	460;500	0.5
Assess rooted cutting vigor prior to outplanting	460;490	0.5
Assess survival of field planted rooted cuttings	490;530	3.0
Select optimum rooting, handling, and delivery systems for cuttings	460;470	1.0
Calculate cost of a rooted cutting	470;480	0.5
Decide whether a clonal reforestation program is feasible for this species	580;590	0.5
Study wood production for pure clonal and mixed clone stands	590;600	45.0
Study tests with varying degrees of genetic diversity and their response to environmental heterogeneity and pest attack	590;610	45.0
Study implications of moving genotypes with different life history strategies[1] to new environments - stability	590;620	45.0
Study implications of mixing genotypes with different life history strategies in the same environment (including inter- and intra-clone competition)	590;630	45.0
Improve and verify biological model developed earlier	640;650	2.0
Use biological model to match genetic components of population to each stand environment	650;660	1.0

[1]Life history strategy in this context includes phenology, slope of growth curve, crop or competitive ideotype, harvest index, etc.

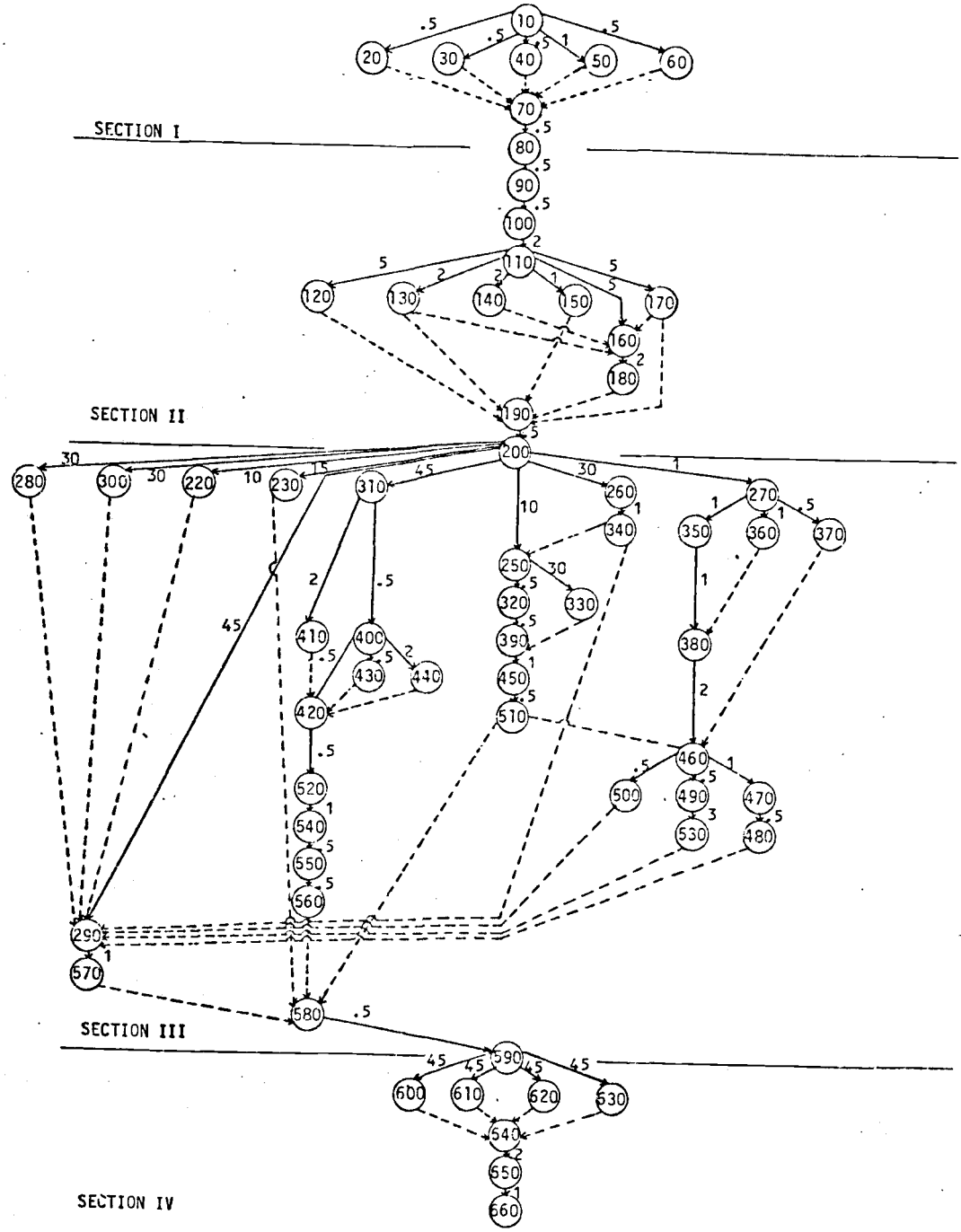

Figure 1. Network diagram of research and development activities supporting a clonal reforestation program. Activities, represented by a line connecting two nodes, are described in Table 1. The number on each activity line is the time (years) required to accomplish each activity.

first four activities in the diagram can be resolved without prior information from any later experiment or analysis.

In the diagram, an opportunity is provided to screen each candidate tree species before conducting lengthy and costly activities. The network diagram was divided into four sections with decision points (to continue or discontinue the project) in between. Starting at the beginning of the diagram (Figure 1), the sections increase in time duration and cost as you proceed down the diagram.

Time estimates were then calculated for each of the four sections as well as for the entire project. Duration times of activities along a path are additive, therefore the summation of the durations along a path yields the duration of the entire path. From these time estimates, the critical path in the diagram was identified.

Evaluating and modifying the model was the final step. Due to the complexity of the model, several revisions were made; and realistically, the model will continue to be modified as it is used. Since the network diagram was not actually implemented, project monitoring cannot be done.

Discussion of Network Analysis

The network divides naturally into four distinct sections with decision points between sections. Cost, duration, and complexity of the activities generally increase from one section to the next. At each decision point, the program manager can evaluate the information to date and determine whether to continue with the research program. By faithfully following network paths, costly long-term studies will

not be started unless they are preceded by favorable economic and biological results from prerequisite activities.

The first section entails species choice. Here, economic and silvicultural information is combined with preliminary information on genetic variation and rooting ability, with an objective of picking candidate species. Species enter the analysis individually and are retained or dropped in activity 70;80 (Figure 1). If at this point, no suitable species is found, the process stops, and only approximately one year's effort will have transpired. If, however, one or more species are found with strong potential for a rooting program, the research program continues.

In the next section, results from a series of research activities, primarily involved with rooting ability and the early performance of rooted cuttings, will make it possible to decide whether to continue the program with one or more species. There is no limit (except financial) to the number of species entering this or any other section, but the addition of a second or third species reduces costs very little per species (few if any economies of scale). Research on each candidate species advances through the diagram independently. The establishment of rooting facilities to refine rooting procedures contributes the most expensive part of this section; but starting pilot-scale field experiments before developing economic rooting procedures could be far more costly. This section also includes a preliminary economic analysis. The section would take approximately 10.5 years to complete and ends with a second major decision point on whether or not to continue the program. Up to this point, the investment in time and money has been relatively modest, yet the decision on

whether to continue the program can be soundly based on both biological and economic evidence.

The third section embraces many activities, some demanding considerable expense and time (Figure 1, Table 1). It should not be entered unless previous research has indicated a high probability of commercial success using clonal reforestation of the species. This section includes research and development concerning tree breeding, growth and yield, clonal multiplication, rooting physiology, growth characteristics of seedlings versus cuttings, and economic analysis. With western hemlock as the example, this section would take about 50 years to complete. Growth studies constitute most of the time, other activities usually being short term. For measuring growth responses, experiments are carried to 2/3 or full rotation (45 years).

The third section also emphasizes an important line of research for which there is little guarantee of success; but if successful, it would greatly shorten the period for evaluating clones and thus would shorten the critical path through this and the final section. The proposed research is to develop a model combining information about genotypes and environments from short-term tests to predict performance of rooted cuttings at later stages in the rotation. Burdon (1971) took steps in this direction using field trials of cuttings of radiata pine (Pinus radiata D. Don), but a successful model probably must incorporate some application of yield-density theory (Drew and Flewelling, 1977; Wearstler, 1979). Such a model would serve two purposes: (1) to shorten growth testing and (2) to investigate matching of clones or clonal mixtures with planting sites (take advantage of genotype x environment interaction).

Economic analysis in the third section primarily addresses costs of clonal versus seedling regeneration (need for artificial reforestation is assumed). Here, as at previous decision points, a poor benefit-cost ratio would provide reason for discontinuing the research.

The fourth section incorporates activities related to adaptability of clones to the plantation environment. For most species, little is known of long-term adaptability of clones to environments variable in both space and time. This information would undoubtedly play an important role in the successful deployment of clones in a reforestation program. In addition, the development of clonal mixtures which include complementary clones would provide for maximum yield per unit area. Each of the four long-term activities (590;600, 590;610, 590;620, 590;630) would undoubtedly include more than one experiment. The biological model developed in the preceding section would supply a means to model several of these activities prior to establishing long-term field tests as well as for shortening the 48.5 years projected for completing this section.

The critical path for this research and development program requires 110.0 years for completion given the 45 year rotation length for the example species, western hemlock. For this species, either deleting activities (incurring risk) or using the proposed biological model to shorten the testing periods would shorten the critical path.

The network analysis pertains only to the acquisition of information. At some stage, depending on results, the research program may combine with a tree improvement program which uses the information. The user then must decide if and when the information is sufficiently

favorable to justify starting clonal reforestation. Any results of studies coming after this would be applied to the clonal program.

Conclusion

Network diagraming holds promise for logically ordering the large number of research and development activities required to implement a clonal reforestation program. Subdividing the activities into four increasingly complex sections with decision points between provides a useful technique to keep exploratory research costs low until there is sufficient evidence of eventual success to proceed. The research and development program described in this analysis can provide information to a reforestation manager who, based upon the evidence provided, would then be able to decide if and when to initiate operational clonal reforestation with a chosen species.

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CHAPTER III. HERITABILITY, GAIN AND C EFFECTS IN ROOTING OF WESTERN HEMLOCK CUTTINGS

Introduction

Forest managers worldwide are interested in clonal reforestation due to its potential for substantial genetic gain in growth traits. Clonal variation in growth traits can be large (Kleinschmit, 1974) and may be due to several factors. In addition to genetic differences, C effects can be a major source of inter-clone variation (Wilcox and Farmer, 1968). C effects are environmental effects that are common to members of a group of relatives such as a clone or a family (Lerner, 1958). These effects persist for various lengths of time (Libby and Jund, 1962; Cannell et al., 1978) and cause the variance among groups to be an over-estimate of the genetic variance. Two types of maternal effects, "M" and "m," have been described (Burdon and Shelbourne, 1974). M is the "maternal effect common to all ramets of a clone" and represents true C effects. m is the maternal effect related to the individual propagule (or cutting) and adds to the variance within but not among clones; therefore m is not really a C effect.

If C effects are large, estimates of total genetic variation are inflated by the C effects variation. The biased estimates of genetic variation in turn influence estimates of genetic parameters such as heritability, correlations among traits, and gain. Over-estimation of genetic variance is a problem particularly in traits measured shortly after cloning (Libby and Jund, 1962). Over-estimation of predicted gain may cause unduly optimistic expectations for a clonal reforestation program. Estimated genotypic values of clones are

biased (Libby and Jund, 1962), resulting in a poor correlation between actual and predicted performance. Tree improvement programs typically require large financial investments which might be unwarranted if actual gain is substantially less than expected.

Most clonal testing or reforestation programs make no attempt to separate genetic and C effects. In this paper, I will examine variation in rooting ability among clones of western hemlock and will estimate for several rooting traits the proportion of variation under genetic control and the proportion due to C effects.

There are many possible causes for C effects in rooting traits of forest trees. Rooting ability of cuttings generally decreases as the age of an ortet increases (Brix and van den Driessche, 1977). The rate of this decrease varies among tree species (McAlpine and Jackson, 1959; Roulund, 1973; Foster, Martin, and Caldwell, 1981) and is generally irreversible (Olesen, 1978) unless some action is taken to control the aging process. Hedging trees, for example, may halt or at least delay the negative effects of aging on rooting ability (Libby, Brown, and Fielding, 1972). Rooting ability is also influenced by the crown position from which cuttings originate. In a Norway spruce [Picea abies (L.) Karst.] study, Roulund (1973) found an average increase in rooting of 2.5 percent per whorl as cutting origin changed from the top to the bottom of the crown. In addition, physiological condition of the donor plant (ortet or ramet) profoundly influences the rooting of cuttings. Dormling and Kellerstam (1981) observed a substantial positive relationship between rooting ability and the vigor of the donor plant. Other factors that can affect rooting ability include cutting size, position of the cutting on the donor tree's

branch (Ying and Bagley, 1977), and presence or absence of reproductive organs on cuttings (Burdon and Shelbourne, 1974).

To my knowledge, partitioning of clonal variation into genetic and C effects components has been reported in only one previous study of forest trees. Wilcox and Farmer (1968) noted that variation due to C effects in cottonwood (Populus deltoides Bartr.) accounted for 9 to 11 percent of the total clonal variation and was one-third the size of genetic variation for two rooting traits. The authors concluded that C effects of this magnitude might cause considerable bias in estimating heritabilities and gains. They suggested that efforts be made to either minimize or control variation due to C effects in clonal reforestation programs.

Several researchers have reported on clonal variation in rooting of western hemlock (hereafter referred to as hemlock) cuttings. Brix and Barker (1975) noted a tremendous amount of variation in rooting ability among trees (ortets) within stands; in one stand, rooting of cuttings ranged from 0 to 100 percent among trees. Foster, Martin, and Caldwell (1981) conducted rooting studies on a juvenile (6-20 years old) and a mature population (19-60 years old) of hemlock. Broad-sense heritabilities for proportion of rooted cuttings per ortet were 0.53 in juvenile and 0.55 in mature populations and for number of main roots per rooted cutting, were 0.31 in juvenile and 0.27 in mature populations. Correlations (product-moment) of the two traits with age of ortets were calculated separately for the two populations. In all cases, the correlations were significant but ranged between -0.14 and -0.21, indicating a small decline of rooting ability with

increasing ortet age. Sorensen and Campbell (1980) estimated broad-sense heritability as 0.23 and narrow-sense heritability as 0.04 for the percent of rooted cuttings per ortet. Open-pollinated families of one-year-old hemlock seedlings were used as ortets. Because non-additive genetic variance was four times the size of additive variance, they concluded that non-additive genetic effects were primarily responsible for the inheritance of rooting ability in hemlock. The proportion of the clonal variance due to C effects was not estimated in either of these hemlock studies. But, C effects, if present, may have inflated estimates of non-additive genetic variance (Sorensen and Campbell, 1980).

Heritabilities have been estimated for several other rooting traits in tree species other than hemlock. Wilcox and Farmer (1968) calculated broad-sense heritabilities of 0.33 or 0.52 (depending on rooting media) for length of the longest main root using cottonwood cuttings. Broad-sense heritabilities in shortleaf pine (Pinus echinata Mill.) have been reported as 0.43 for length of longest main root and 0.46 for the sum of the lengths of all main roots (Fancher and Tauer, 1981).

This study had four objectives: (1) provide information to the network analysis (Chapter II) of research and development for clonal reforestation with hemlock, (2) determine possible sources of C effects when rooting cuttings from field-grown ortets (Experiment 1) (110;170)[1], (3) test for the presence and size of C effects in rooting ability when attempts are made to decrease C effects (Experiment 2)

[1]Numbers in parenthesis refer to activities in the network analysis in Chapter II which are advanced by these experiments.

(160;180), and (4) estimate heritabilities and expected gains for rooting traits of hemlock cuttings as well as genetic correlations among traits and potential correlated responses to selection (Experiment 2) (110;160).

Materials and Methods

Experiment 1

To test the effects of crown position of field ortets on rooting ability, four hemlock trees were chosen at random from stands near Seaside, Oregon. A long, full crown and a minimum age of 25 years were the only selection criteria. Tree age at stump height ranged from 29 to 65 years (Table 2).

Cuttings were collected from felled trees on December 15, 1980. Before felling the directions, North, South, East, and West, were marked on the trees. Trees were then felled, and crowns on down trees were divided into four vertical sections of equal-length for sampling purposes. Ring counts were taken at the bottom of each section for determining age (Table 2). Twenty-five stem cuttings from the current-year's growth were collected at random from the northeast and southwest facing quadrants (aspects) within each of the four crown positions. Cuttings thus came from eight distinct sample locations (2 aspects x 4 positions) in each tree.

The cuttings were set in a rooting chamber on December 22, 1980. Prior to setting, each cutting was reclipped to a standard length of ten cm when possible. The majority of the cuttings met the standard length ($\bar{x}$ = 9.4 cm and range of 3.5 to 12.0 cm). Needles were removed

Table 2. Crown section ages (years)[1] for the four hemlock trees in Experiment 1.

Tree Number	Section 4	3	2	1
1	29	18	14	9
2	30	19	13	9
3	65	31	21	9
4	37	25	18	13
Average	40.3	23.3	16.5	10.0

[1]Each tree was divided into four, equal-length sections and age (ring count) was taken at the base of each section; section height increases with decreasing section number. Age of section 4 was taken at stump height.

from the lower two cm of the cutting base. Cuttings were then quickly dipped into a 0.15 gm/l solution of Benomyl and water, lightly tapped to remove excess solution, and dipped (lower two cm of cutting base) into an auxin solution. The auxin solution contained "Dip'n Grow" (C-R Chemical Research Co., Portland, OR) and distilled water in a 1:10 ratio. The active ingredients in undiluted "Dip'n Grow" were 1.0 percent IBA (Indole-3-butyric acid), and 0.5 percent NAA (1-Naphthaleneacetic acid).

Treated cuttings were set in media-filled plastic containers (65 ml) for rooting. The medium included a 1:1:1 ratio of shredded peat, perlite, and coarse sand and was steam sterilized before use.

The rooting chamber consisted of a wood-framed, vinyl tent measuring 16.9 m long, 1.2 m wide, and 0.8 m high. A single chamber was used and was located inside a larger greenhouse. High humidity (average 80 percent relative humidity) was maintained within the chambers by using an intermittent, misting system which produced a dense fog of water. CO_2 and air were injected into the nozzle heads to produce the fog. The misting system was controlled by a combination of manual and automatic controls; when operating, a timer engaged the misting system for 1 1/2 to 2 minutes on a 12 minute cycle. Very little misting was required when the ambient greenhouse temperature was below 15°C. The humidity was monitored and the mist system was manually turned on when needed until the humidity reached the desired level. Once the greenhouse temperature rose above 15°C a thermostatically-controlled system took over to maintain the temperature below 21°C and the relative humidity above 80 percent.

Ambient air from inside the greenhouse was pumped into the chamber to help control both temperature and humidity (the greenhouse was artificially cooled in the summer). The temperature inside the chamber was maintained as follows: 10-13°C December-April, 15-20°C May-August. Day length was kept at 16 hours throughout the experiment by using artificial lighting.

The cuttings were treated weekly with an application of fertilizer and fungicide. The fertilizer was a balanced 20-20-20 foliar spray at 150 ppm, applied each Monday with an application of 50-75 ppm kelated iron every two weeks; the fungicide was a foliar spray applied each Friday and rotated weekly among 1800 ppm Botran, 181 ppm Captan, and 1190 ppm Daconil.

After seven months in the rooting chamber, the cuttings were lifted and evaluated on August 13, 1981. Five traits related to rooting ability were measured on the cuttings in this experiment: cuttings per five-cutting plot (RC), length of the longest main root on each rooted cutting (LTH), number of main roots (roots beginning at the cutting base) per rooted cutting (MR), sum of the lengths of all main roots per cutting (VOL), and number of quadrants (of the cutting base) from which main roots originate per rooted cutting (because the cutting base was divided into four equal quadrants, possible values for each cutting were 1 to 4) (QD). VOL is an index of root system volume. RC expresses the overall ability of a clone to form roots on its cuttings and included zero-values. LTH, MR, VOL, and QD reflect the quality of the developing root system, are measured only on rooted cuttings, and therefore cannot be zero.

The experimental design was a randomized complete block with five blocks. Each plot consisted of a row of five cuttings from one crown location of one sampled tree. The blocks were arranged along the gradient of mist distribution. The form of the analysis of variance is presented in Table 3. Crown positions and aspects were considered to be fixed effects while blocks and clones were considered to be random. The sum of squares due to crown positions and their interactions with aspects and clones were further partitioned into linear and quadratic trends.

The analysis varied somewhat among the five traits. Since RC represented a plot value, it was analyzed in its original form. Plot means, however, were calculated and analyzed for each of the other four traits: LTH, MR, VOL, and QD. Due to an error in the original test installation, four plots were deleted from block number one and missing values were estimated (using Winer, 1971, p. 489) for the analysis. Six plots had zero rooting; therefore for RC, these plots received a value of zero for the analysis. For the other four traits, additional missing values (due to plots with zero rooting) were estimated using the same technique as above. Degrees of freedom in the analysis of variance were adjusted for the number of missing values. Coefficients of the variance components (Table 3) were adjusted (Searle, 1971) to compensate for missing values which were estimated. The data for each trait were examined for compliance with assumptions for analysis of variance (Sokal and Rohlf, 1969). Data for two traits, VOL and QD, were transformed using the $\log_{10}$ transformation due to heterogeneous treatment variances. The data for the other three traits did not require transformation.

Table 3. Form of analysis of variance for rooting traits of hemlock clones with cuttings taken from varying crown positions and crown aspects.

Source of variation	Degrees of freedom	Expected mean squares[1]
Blocks (B)	4	$\sigma^2 + cpa\sigma^2_B$
Clones (C)	3	$\sigma^2 + bpa\sigma^2_C$
Crown positions (P)	3	$\sigma^2 + ba\sigma^2_{CP} + bca\theta^2_P$
Crown aspects (A)	1	$\sigma^2 + bp\sigma^2_{CA} + bcp\theta^2_A$
C x P	9	$\sigma^2 + ba\sigma^2_{CP}$
C x A	3	$\sigma^2 + bp\sigma^2_{CA}$
A x P	3	$\sigma^2 + b\sigma^2_{CPA} + bc\theta^2_{PA}$
C x A x P	9	$\sigma^2 + b\sigma^2_{CPA}$
Error	124	σ^2

[1] b, c, p, and a were, respectively, the number of blocks, clones, crown positions, and crown aspects.

σ^2_B = variance among blocks.

σ^2_C = variance among clones.

θ^2_P = effects due to crown position differences.

θ^2_A = effects due to crown aspect differences.

σ^2_{CP} = variance due to interaction of clones and crown positions.

σ^2_{CA} = variance due to interaction of clones and crown aspects.

θ^2_{PA} = variance due to interaction of crown positions and aspects.

σ^2_{CPA} = variance due to interaction of clones, crown positions, and aspects.

σ^2 = error variance.

Experiment 2

The second experiment investigated clonal variation in rooting ability using 60 hemlock clones, sampled from a wide area. The ortets of these clones were a randomly chosen subset of the select trees included in a low-intensity, comparison-tree selection program. Geographically, the ortets were located along road systems on Crown Zellerbach Corporation Managed Forests between Tillamook, Oregon (45° 30' N latitude) and Clallam Bay, Washington (48° 15' N latitude) in the Coast Ranges between 0 and 300 m in elevation. Ortets ranged in age from 28 to 65 years at d.b.h.

The 60 ortets (and clones) are assumed to be a random sample from the wild populations. Select trees were not chosen for rooting characteristics, and analysis of fifth-year heights from progeny tests of all select trees (Foster and Lester, 1983) indicated no significant difference from random seed collections.

Stem cuttings of current-year's growth were collected from the top one-third of the crown of parent trees in January, 1976, and rooted in mist benches during the year. The rooted cuttings from each ortet were potted in February, 1977, and allowed to grow freely in a greenhouse for two growing seasons. In the late winters of 1978 and 1979, ramets (primary ramets) from each of the 60 clones were hedged to maintain a low bush--shorter than 0.5 m. All ramets were grown in the same size pots and same potting medium in a greenhouse under uniform environmental conditions.

On December 17 and 18, 1980, current-year's stem cuttings were removed from three hedged primary ramets of each of the 60 clones and

set (December 23 and 24, 1980) in a mist bench for rooting. These cuttings were subsequently treated the same as those in Experiment 1. The cuttings were lifted and evaluated from July 7 to July 16, 1981. The overall scheme of this experiment is illustrated in Figure 2.

The experimental design consisted of a randomized complete block layout with 8 blocks, 60 clones, and 3 primary ramets per clone. Each plot consisted of a row of five cuttings from a single primary ramet. In total there were 180 (60 x 3) plots per block.

The form of the analysis of variance is presented in Table 4. The same five traits were measured in Experiment 2 and in Experiment 1. Using a least-squares procedure, the data from trait RC were analyzed as individual measurements, and for the other four traits, plot means were analyzed. The actual coefficients of the variance and covariance components (Table 4) were adjusted to compensate for some missing plots (Searle, 1971). The data for each trait were checked for agreement with the assumptions of analysis of variance (Sokal and Rohlf, 1969); data transformation was not required for any of the traits. All sources of variation were considered to be random.

Components of variance were calculated by equating observed mean squares to expected mean squares and solving the resulting equations. Broad-sense heritabilities were estimated for each of the five traits. Since genotypic values of clones are estimated by the average performance of the ramets, broad-sense heritability is calculated on a clone-mean basis (Bridgwater, 1972). Individual-tree heritability is used only if individual ramets are selected. The broad-sense heritability was calculated as:

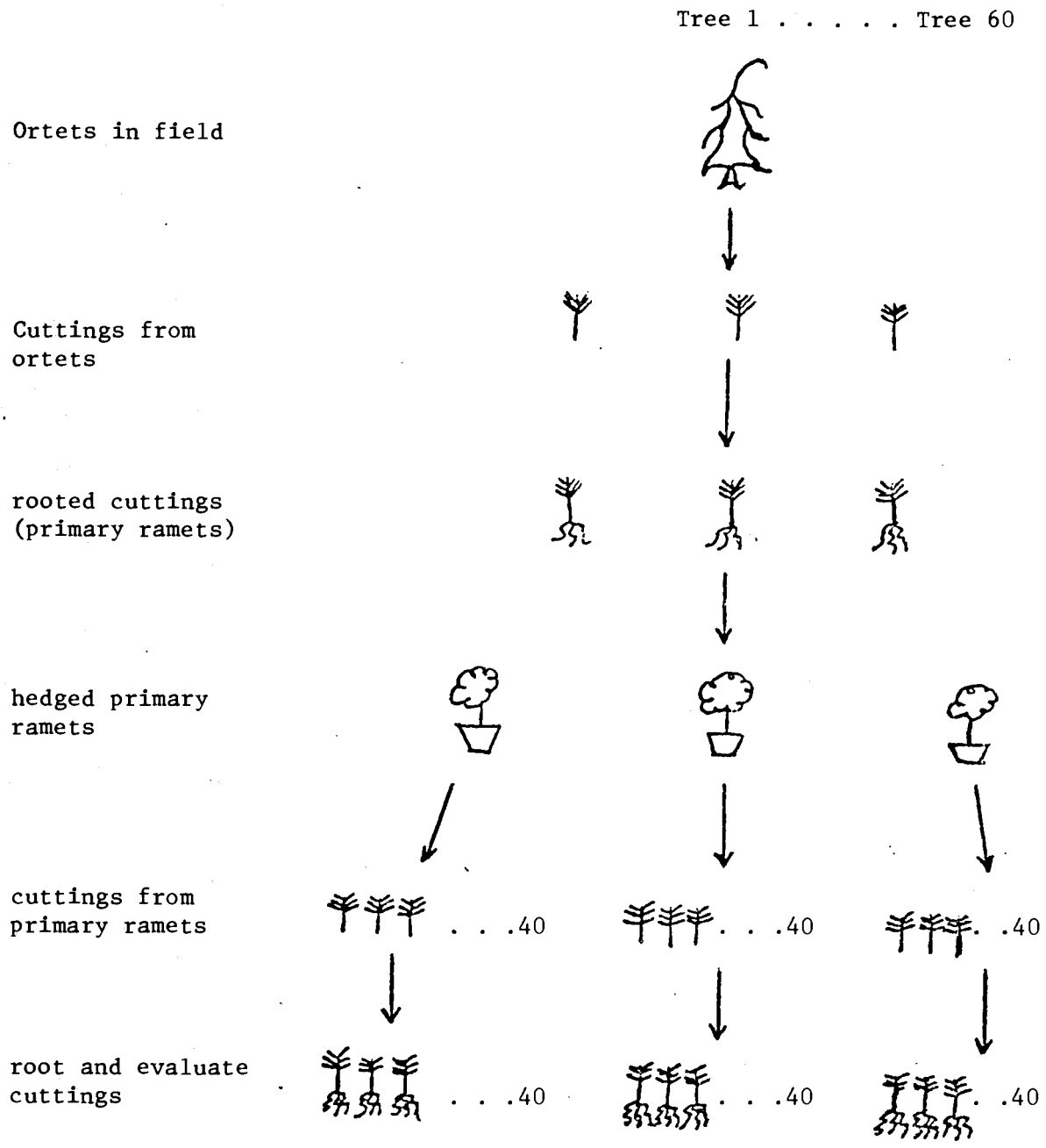

Figure 2. Secondary cloning process of hemlock trees for Experiment 2.

Table 4. Form of analysis of variance of rooting traits in primary ramets of hemlock clones.

Source of variation	Degrees of freedom	Expected mean squares[1]
Blocks (B)	7	$\sigma^2 + pc\sigma^2_B$
Clones (C)	59	$\sigma^2 + b\sigma^2_{P(C)} + bp\sigma^2_C$
Primary ramets (P)/C	120	$\sigma^2 + b\sigma^2_{P(C)}$
Error	1253	σ^2

[1] b, p, and c were the number of blocks of each clone, primary ramets per clone, and clones per block, respectively.

σ^2_B = variance among blocks

σ^2_C = variance among clones.

$\sigma^2_{P(C)}$ = variance among primary ramets within clones.

σ^2 = error variance.

$$H = \frac{\hat{\sigma}^2_C}{\hat{\sigma}^2_C + \frac{\hat{\sigma}^2_{P(C)}}{p} + \frac{\hat{\sigma}^2}{pb}}$$

where symbols are as defined in Table 4.

Expected genetic gain was estimated for response of rooting traits to clonal selection. Gain for trait k, (G_k), was calculated using the following equation:

$$G_k = iH_k\hat{\sigma}_{X_{(k)}}$$

where: i = selection intensity (10 percent (i = 1.755) was assumed)

H_k = broad-sense heritability of trait k

$\hat{\sigma}_{X_{(k)}}$ = phenotypic standard deviation of trait k

$$= \left(\hat{\sigma}^2_C + \frac{\hat{\sigma}^2_{P(C)}}{p} + \frac{\hat{\sigma}^2}{pb}\right)^{1/2} \text{ of trait k}$$

Genetic and phenotypic correlations among the six traits were also estimated using the technique of Johnson, Robinson, and Comstock (1955). In order to calculate correlations between RC and any of the other four traits, all zero-valued plots for RC were deleted for correlation purposes. Genetic correlation was calculated as:

$$\hat{r}_{g(X,Y)} = \frac{\hat{\sigma}_{C(X,Y)}}{\hat{\sigma}_{C(X)}\hat{\sigma}_{C(Y)}}$$

where: $\hat{r}_{g(X,Y)}$ = genetic correlation between traits X and Y

$\hat{\sigma}_{C(X,Y)}$ = clonal component of covariance between traits X and Y

$\hat{\sigma}_{C(X)}$ = square root of clonal component of variance for trait X

$\hat{\sigma}_{C(Y)}$ = square root of clonal component of variance for trait Y

The phenotypic correlation between traits was calculated as:

$$\hat{r}_{P(X,Y)} = \frac{MCP_{C(X,Y)}}{(MS_{C(X)}MS_{C(Y)})^{1/2}}$$

where: $\hat{r}_{P(X,Y)}$ = phenotypic correlation between traits X and Y

$MCP_{C(X,Y)}$ = clonal mean cross product between traits X and Y

$MS_{C(X)}$ = clonal mean square for trait X

$MS_{C(Y)}$ = clonal mean square for trait Y

The magnitude of the standard errors of the correlations provides a measure of their precision. Since the distribution of these correlations is unknown (Scheinberg, 1966), tests of significance assuming a normal distribution should be used with caution. The approximate variances of genetic and phenotypic correlations were estimated (McCullough, 1968).

If,

$$\hat{r}_a = \frac{\Theta_{12}}{(\Theta_{11}\Theta_{22})^{1/2}}$$

then,

$$\text{var}(\hat{r}_a) = \hat{r}_a^2 \left[\frac{\text{Var}(\theta_{12})}{\theta_{12}^2} + \frac{\text{Var}(\theta_{11})}{4\theta_{11}^2} + \frac{\text{Var}(\theta_{22})}{4\theta_{22}^2} - \right.$$

$$\left. \frac{\text{Cov}(\theta_{12}, \theta_{11})}{\theta_{11}\theta_{12}} - \frac{\text{Cov}(\theta_{12}, \theta_{22})}{\theta_{22}\theta_{12}} + \frac{\text{Cov}(\theta_{11}, \theta_{22})}{2\theta_{11}\theta_{22}} \right]$$

where symbols are explained in Table 5. Standard errors of the correlations are estimated by taking the square root of variances.

If two traits are genetically correlated, selection for one trait yields a correlated response in the other. Predicted correlated responses were calculated using the technique of Falconer (1960):

$$CR_y = i\sqrt{H_x}\ \sqrt{H_y}\ \hat{r}_{g(x,y)}\ \hat{\sigma}_{x(y)}$$

where: i = selection intensity (10 percent ($i = 1.755$) was assumed)

H_x = broad-sense heritability of trait X

H_y = broad-sense heritability of trait y

$r_{g(x,y)}$ = genetic correlation between traits X and Y

$\hat{\sigma}_{x(y)}$ = phenotopic standard deviation of trait y

The results of this experiment provided estimates of variance components which can be used to help improve the design of future rooting experiments. Because the clone mean is the basis of selection and gain, the variance of a clone mean is of particular interest; it reflects the precision with which the means are estimated. Also the variance of a clone mean, $V_{\overline{X}}$, is an intergal part of the phenotypic variance of clone means, $\sigma^2_{X_{(k)}}$, and the phenotypic standard deviation

Table 5. Modified equations used to estimate the variance of genetic and phenotypic correlation coefficients.

Parameter	a = genetic[1]	a = phenotypic
Θ_{12}	$\frac{MCP_{C(X,Y)} - MCP_{P(X,Y)}}{k}$	$\frac{MCP_{C(X,Y)}}{k}$
Θ_{11}	$\frac{MS_{C(X)} - MS_{P(X)}}{k}$	$\frac{MS_{C(X)}}{k}$
Θ_{22}	$\frac{MS_{C(Y)} - MS_{P(Y)}}{k}$	$\frac{MS_{C(Y)}}{k}$
$Var(\Theta_{12})$	$\frac{MS_{C(X)}MS_{C(Y)} + MCP^2_{C(X,Y)}}{k^2(df_C + 2)} + \frac{MS_{P(X)}MS_{P(Y)} + MCP^2_{P(X,Y)}}{k^2(df_P + 2)}$	$\frac{MS_{C(X)}MS_{C(Y)} + MCP^2_{C(X,Y)}}{k^2(df_C + 2)}$
$Var(\Theta_{11})$	$\frac{2MS^2_{C(X)}}{k^2(df_C + 2)} + \frac{2MS^2_{P(X)}}{k^2(df_P + 2)}$	$\frac{MS^2_{C(X)}}{k^2(df_C + 2)}$
$Var(\Theta_{22})$	$\frac{2MS^2_{C(Y)}}{k^2(df_C + 2)} + \frac{2MS^2_{P(Y)}}{k^2(df_P + 2)}$	$\frac{MS^2_{C(Y)}}{k^2(df_C + 2)}$
$Cov(\Theta_{12}, \Theta_{11})$	$\frac{2MS_{C(X)}MCP_{C(X,Y)}}{k^2(df_C + 2)} + \frac{2MS_{P(X)}MCP_{P(X,Y)}}{k^2(df_P + 2)}$	$\frac{2MCP_{C(X,Y)}MS_{C(X)}}{k^2(df_C + 2)}$

Table 5 (continued)

Parameter	a = genetic[1]	a = phenotypic
$Cov(\Theta_{12}, \Theta_{22})$	$\frac{2MS_{C(Y)}MCP_{C(X,Y)}}{k^2(df_C + 2)} + \frac{2MS_{P(Y)}MCP_{P(X,Y)}}{k^2(df_P + 2)}$	$\frac{2MCP_{C(X,Y)}MS_{C(Y)}}{k^2(df_C + 2)}$
$Cov(\Theta_{11}, \Theta_{22})$	$\frac{2MCP^2_{C(X,Y)}}{k^2(df_C + 2)} + \frac{2MCP^2_{P(X,Y)}}{k^2(df_P + 2)}$	$\frac{2MCP^2_{C(X,Y)}}{k^2(df_C + 2)}$

k = number of blocks x number of primary ramets.

df_C = degrees of freedom at clonal level.

df_P = degrees of freedom at primary ramet in clone level.

All other symbols are explained in text.

[1]McCullough, 1968; personal communication from Dr. T. White, Forest Geneticist, International Paper Co., Lebanon, Oregon.

of clone means is used to calculate broad-sense heritability (H) and predicted gain (G). The theoretical variance of a clone mean is:

$$V_{\bar{X}} = \frac{\hat{\sigma}^2_{P(C)}}{p} + \frac{\hat{\sigma}^2}{pb}$$

and

$$H = \frac{\hat{\sigma}^2_C}{\hat{\sigma}^2_C + V_{\bar{X}}}$$

since

$$\hat{\sigma}_X = \sqrt{\hat{\sigma}^2_C + V_{\bar{X}}}$$

where symbols are defined in Table 4. To predict results in future experiments the variance components are assumed to be stable while the coefficients, p and b, vary. This technique can be used in two ways: (1) the number of plots (i.e., pb) can be assumed to be fixed and different allocations of p and b can then be examined to find the minimum variance ignoring cost and time (Rasmusson and Lambert, 1961); or (2) the total number of plots can be allowed to vary, and the sensitivity of $V_{\bar{X}}$ to changing allocations of p and b can be examined (Schutz and Bernard, 1967) with its resultant effect on H and G. The second approach was used in this study to predict the effects of altering the experimental design.

Results

Experiment 1

The first experiment sought to indicate possible sources of C effects which might be encountered in the rooting of cuttings from sexually mature hemlock ortets. In addition, the magnitude of potential C effects variation is compared to the size of clonal variation.

Significant variation existed among the four clones for all five of the rooting traits that were measured (Tables 6 and 7). Clone 3 caused much of the variation; it rooted consistently poorer, based on all five traits, than the other three clones. Clone 2, on the other hand, rooted as well or better than the other clones.

In addition to clonal differences, variation due to crown position was significant for RC, LTH, VOL, and QD (Table 7). In general, rooting ability was lowest at the top of the crown and increased toward the bottom (Figure 3). A strong linear trend for crown positions occurred for all five traits and explained a significant amount of the sum of squares due to crown positions (Table 7). The sum of squares for the linear trend accounted for 99 (RC), 97 (LTH), 71 (MR), 95 (VOL), and 77 (QD) percent of the among-positions sum of squares. Neither quadratic trends nor deviations from linear and quadratic trends for crown positions were significant for any of the five traits.

Differences in rooting ability due to the two crown aspects were significant only for RC (Table 7). For this trait, cuttings taken

Table 6. Average values[1] (standard errors) of five rooting traits for four hemlock clones.

Clones	Rooted cuttings per plot (RC)	Main roots (MR)	Quadrants with roots (QD)	Length longest main root (LTH)	Sum of length of main roots (VOL)
	(number)			(cm)	
1	3.5 (0.19)	2.9 (0.17)	2.2 (0.12)	4.9 (0.30)	8.3 (0.62)
2	3.9 (0.17)	3.3 (0.13)	2.4 (0.08)	4.8 (0.20)	9.6 (0.48)
3	1.9 (0.19)	1.4 (0.07)	1.3 (0.07)	2.8 (0.25)	3.4 (0.33)
4	3.8 (0.19)	2.8 (0.15)	2.0 (0.09)	3.6 (0.24)	6.3 (0.44)

[1]Mean of untransformed data.

Table 7. Analysis of variance for five rooting traits of hemlock cuttings.

Source of variation	Mean squares				
	RC	LTH	MR	log(VOL+1)	log(QD+1)
Blocks (B)	4.58*	13.87**	3.35**	0.26**	0.028**
Clones (C)	35.01**	41.25**	27.72**	1.09**	0.198**
Crown positions (P)	11.76**	11.45*	4.85^{NS}	0.26*	0.051*
Linear	34.86**	33.35**	10.34*	0.74**	0.118*
Quadratic	0.31^{NS}	0.19^{NS}	0.70^{NS}	0.00^{NS}	0.001^{NS}
Deviations	0.10^{NS}	0.80^{NS}	3.50^{NS}	0.03^{NS}	0.034^{NS}
Crown aspects (A)	4.56*	8.16^{NS}	8.37^{NS}	0.22^{NS}	0.020^{NS}
C x P	1.41^{NS}	1.97^{NS}	1.58*	0.05^{NS}	0.011^{NS}
Linear	2.33^{NS}	1.96^{NS}	3.28**	0.07^{NS}	0.025*
Quadratic	0.41^{NS}	0.27^{NS}	0.40^{NS}	0.04^{NS}	0.000^{NS}
Deviations	1.50^{NS}	3.67^{NS}	1.06^{NS}	0.04^{NS}	0.009^{NS}
C x A	0.32^{NS}	3.14^{NS}	1.39^{NS}	0.03^{NS}	0.007^{NS}
A x P	0.91^{NS}	0.53^{NS}	0.83^{NS}	0.01^{NS}	0.005^{NS}
Linear	1.36^{NS}	0.02^{NS}	0.02^{NS}	0.00^{NS}	0.000^{NS}
Quadratic	0.16^{NS}	0.31^{NS}	1.30^{NS}	0.00^{NS}	0.011^{NS}
Deviations	1.20^{NS}	1.26^{NS}	1.16^{NS}	0.02^{NS}	0.004^{NS}
C x A x P	1.07^{NS}	2.24^{NS}	0.70^{NS}	0.05^{NS}	0.003^{NS}
Linear	1.92^{NS}	0.51^{NS}	0.98^{NS}	0.01^{NS}	0.004^{NS}
Quadratic	0.99^{NS}	1.12^{NS}	0.49^{NS}	0.10*	0.001^{NS}
Deviations	0.31^{NS}	5.10^{NS}	0.63^{NS}	0.05^{NS}	0.003^{NS}
Error[1]	1.38	2.16	0.77	0.03	0.007

[1]Missing data resulted in smaller actual error degrees of freedom and minor differences in actual E(M.S.). For number of rooted cuttings per plot (RC), error degrees of freedom equal 120; for all other traits, 114.

* Significant at the 5 percent level.

** Significant at the 1 percent level.

^{NS} Not significant at the 5 percent level.

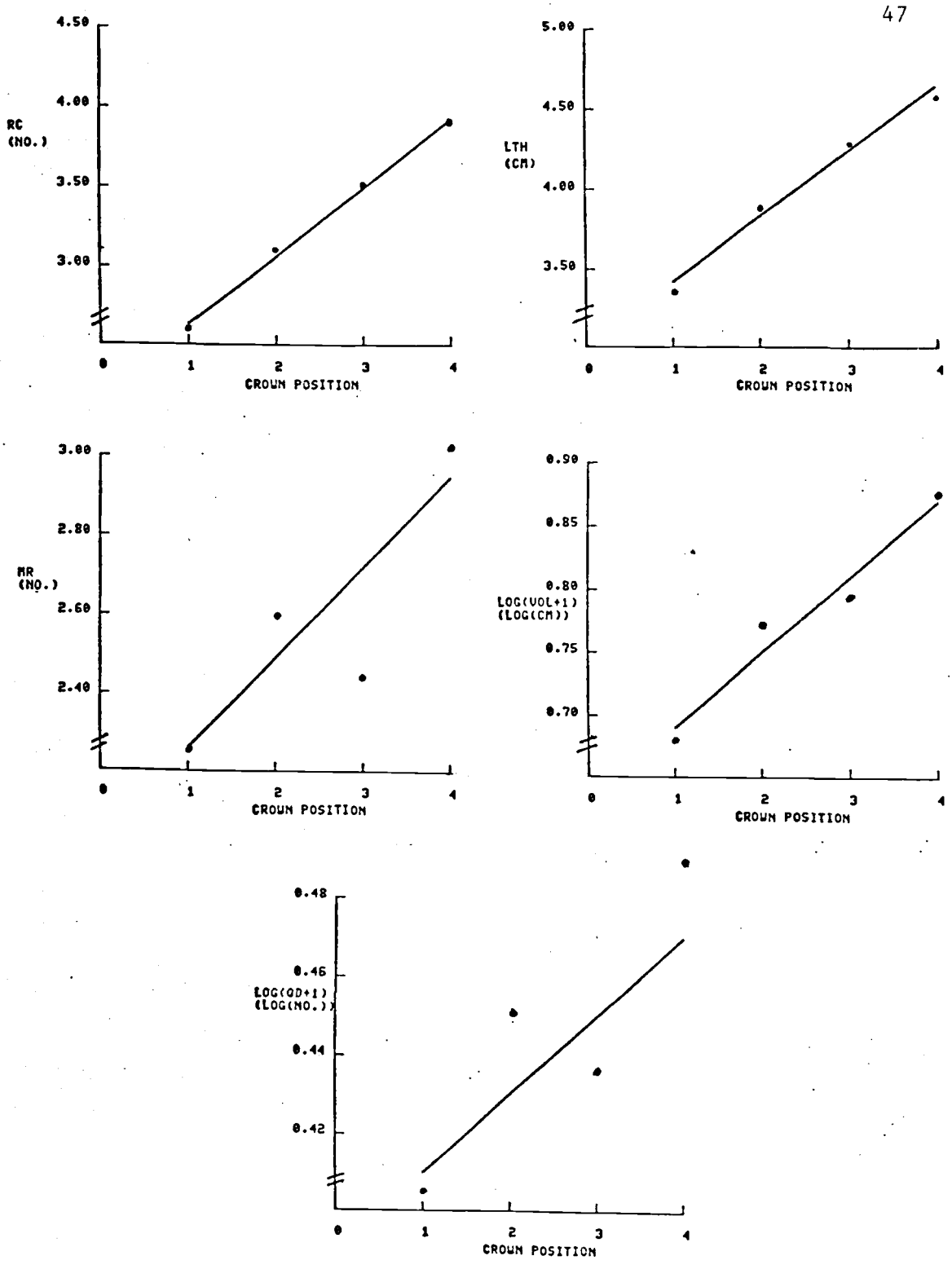

Figure 3. Associations between crown position of cutting collection and rooting traits of hemlock clones.

from the SW aspect rooted slightly better (mean 3.4) than cuttings from the NE aspect (mean 3.1).

Environmental differences within the rooting chamber may cause clones to root less well than indicated by their genetic potential. Significant block effects occurred for all five rooting traits showing that the five traits are indeed sensitive to rooting environment. If all the cuttings from a clone were placed in one location within the rooting chamber instead of being scattered throughout, results will be unduly biased against a clone located in a poor rooting environment.

The main effects in the model accounted for most of the variation present, and few interactions were significant. A significant clone x crown position interaction occurred for MR, and the presence of this interaction may explain the lack of significant average differences among crown positions for this trait (Table 7). Interaction between clones and crown positions for MR (Table 7) can largely be accounted for by different linear trends among clones. Clone 3 was the main cause of the interaction. In contrast to an average increase in MR from the top of the crown to the bottom for all clones combined, MR was low at all crown positions in clone 3. QD also displayed a significantly different linear trend among clones for crown positions. As with MR, clone 3 was the main cause of the differences in linear trends. No other linear or quadratic trends were significant except the quadratic trend for the clone x aspect x position interaction for VOL. Removing the data from clone 3 reduced the quadratic trend to

non-significance, thereby again implicating clone 3 as the cause of interaction.

C effects for rooting ability could arise in a clonal propagation program if care is not taken to collect cuttings from the same location on each ortet. For the five traits, clonal differences accounted for 25 to 37 percent of the total variation in the experiment. In comparison, the sum of differences among crown positions, aspects, and interactions of these traits with clones accounted for 9 to 12 percent of the total variation for the five traits. If these sources of variation were permitted to contribute to C effects, the potential variance among clones due to C effects would be approximately one-third the size of the clonal variance. Obviously, non-genetic variance among clones (due to collecting cuttings from different crown positions among ortets) for rooting traits could substantially bias estimates of genetic parameters.

Experiment 2

The results of the first experiment indicated possible sources and magnitudes of C effects in rooting traits. The second experiment partitioned clonal variation into total genetic variation and C effects. Estimates of genetic variation, heritabilities, and predicted genetic gain from clonal selection were influenced by C effects.

Variation among the 60 clones was significant for all five traits (Table 8). Clonal variation was a large source of variation in the

Table 8. Analysis of variance for estimating influences of clone and C effects on five rooting traits of hemlock cuttings.

Source of variation	Mean squares				
	RC	LTH	MR	VOL	QD
Blocks (B)	23.53**	26.94**	2.66**	101.19**	1.57**
Clones (C)	21.72**	44.59**	15.29**	317.03**	4.28**
Primary ramets (P)/C	2.75**	5.96**	1.16*	31.31**	0.48**
Error[1]	1.55	3.61	0.93	17.90	0.34

* Significant at the 5 percent level.

** Significant at the 1 percent level.

[1]Missing data resulted in some loss of error degrees of freedom. For number of rooted cuttings per plot (RC), error degrees of freedom equal 1211; for all other traits, it equals 1043.

model, accounting for 31 to 42 percent of the total variation (Table 9). Primary ramets-within-clones constituted a significant source of variation for all five traits (Table 8) and accounted for two to six percent of the total variation (Table 9). Differences among primary ramets (hedged ramets) within clones originate entirely from environmental sources and estimate one type of C effects variation (Libby and Jund, 1962; Wilcox and Farmer, 1968).

Estimated heritabilities for the five traits ranged from 0.87 to 0.92 (Table 10) indicating that, in this experiment, observed differences among clones in rooting ability were due almost entirely to genetic effects. Had the variation due to C effects been completely confounded with genetic variation, heritabilities would have been overestimated, with inflated heritabilities of 0.93 (RC), 0.92 (LTH), 0.94 (MR), 0.94 (VOL), and 0.92 (QD). The bias of heritabilities therefore ranged from 0.02 to 0.06, about 2 percent for the trait with highest heritability (MR) and 7 percent for the trait with lowest heritability (RC).

Predicted genetic gains by selecting the top ten percent of the clones (6 out of 60) were substantial due to the generally high heritabilities and large phenotypic standard deviations of the five traits (Table 10). Expected gains for the rooting traits ranged from 37 to 77 percent of the population mean (Table 10). Apparently, one generation of clonal selection for rooting traits could produce substantial changes in population means.

Experimental results indicated that the five rooting traits were closely associated both genetically and phenotypically. Genetic

Table 9. Estimated variance components (standard errors[1]) for five rooting ability traits of hemlock.[2]

Variance component	RC		LTH		MR		VOL		QD	
	Estimate	% total variation	Estimate	% total variation	Estimate	% total variation	Estimate	% total variation	Estimate	% total variation
σ^2_B	0.126 (.063)	5	0.152 (.083)	2	0.011 (.008)	1	0.543 (.311)	1	0.008 (.005)	1
σ^2_C	0.816 (.170)	31	1.900 (.399)	32	0.695 (.137)	41	14.047 (2.829)	41	0.187 (.038)	33
$\sigma^2_{P(C)}$	0.155 (.046)	6	0.347 (.155)	6	0.034 (.023)	2	1.977 (.605)	6	0.020 (.009)	4
σ^2	1.546 (.063)	58	3.606 (.158)	60	0.930 (.041)	55	17.902 (.783)	52	0.343 (.015)	62

[1]Namkoong (1979).

[2]Calculated from observed mean squares in Table 7. Adjusted values of variance components were as follows: b = 7.75 for RC and 6.78 for LTH, MR, VOL, and QD. C = 58.24 for RC and 51.17 for the other traits. P = 3 for all traits.

Table 10. Estimated broad-sense heritabilities (standard error[1]) and potential genetic gains based on clonal selection for five rooting traits of hemlock cuttings.

Trait	Mean ($\overline{X}_{(k)}$)	Phenotypic standard deviation ($\sigma_{X_{(k)}}$)	Range of clone means	Coefficient of variation[3] (%)	Heritability	Genetic gain[2]	
						Measured units	Percent of mean
RC (number)	2.67	0.967	1.0-4.5	36	0.87 (0.03)	1.48	55
LTH (cm)	4.65	1.481	1.1-8.3	32	0.87 (0.03)	2.26	48
MR (number)	2.50	0.867	1.3-4.8	35	0.92 (0.02)	1.40	56
VOL (cm)	8.12	3.948	1.5-20.6	49	0.90 (0.02)	6.24	77
QD (number)	1.90	0.458	1.1-3.0	24	0.89 (0.03)	0.72	37

[1]Namkoong (1979).

[2]Assuming a 10 percent selection intensity, i = 1.755.

[3] $\frac{\hat{\sigma}_{X_{(k)}}}{\overline{X}_{(k)}} (100) = C.V.$

correlations ranged from 0.66 to 0.99 for pairwise combinations of the five traits (Table 11); phenotypic correlations were slightly but consistently smaller and ranged from 0.62 to 0.97.

Since the traits are closely related genetically, direct selection for one trait should theoretically change the population mean for other traits. In the past, researchers have been mainly concerned with percent rooting (RC) of clones. Direct selection for RC should produce large correlated responses in the other four traits (Table 12). In fact, the predicted genetic gains for the other traits are only slightly smaller than the predicted gains if traits are directly selected (Table 10). MR is the trait with the highest heritability (Table 10) and is also strongly correlated with the other four traits (Table 11). Direct selection for MR yields a different set of gain predictions than direct selection for RC. On the average, correlated responses are larger (Table 12) when selecting for MR.

Variance component estimates indicate that the rooting design (five cuttings per plot per block, eight blocks, and three ramets per clone) may have been unnecessarily precise. The goal of a design for estimating clone means is to minimize the variance of a clone mean ($V_{\bar{X}}$) for a given cost. The variance of a clone mean is sensitive to the number of primary ramets and blocks used in the experiment, with the variance decreasing as ramets and blocks increase (Figure 4). This is balanced against the cost of including additional primary ramets (i.e., maintenance of the ramets and cost of added record keeping and of producing more cuttings) and blocks (cost of plot layout and setting more cuttings); so a compromise involving both

Table 11. Phenotypic (above diagonal) and genetic (below diagonal) correlations and their standard errors (in parentheses) among five rooting traits of hemlock cuttings.[1]

Trait	Trait				
	RC	LTH	MR	VOL	QD
RC		0.62 (0.08)	0.83 (0.04)	0.76 (0.05)	0.84 (0.04)
LTH	0.70 (0.09)		0.63 (0.08)	0.92 (0.02)	0.63 (0.08)
MR	0.91 (0.04)	0.66 (0.08)		0.86 (0.03)	0.97 (0.01)
VOL	0.85 (0.05)	0.93 (0.02)	0.87 (0.03)		0.83 (0.04)
QD	0.93 (0.04)	0.67 (0.09)	0.99 (0.01)	0.87 (0.04)	

[1]All phenotypic correlations are significant at the 1 percent level.

Table 12. Correlated responses for five rooting traits of hemlock cuttings, given direct clonal selection for RC or MR.

Trait	Selection for RC		Selection for MR	
	Correlated response[1]		Correlated response	
	Measured units	Percent of mean	Measured units	Percent of mean
RC (number)	(1.48)	(55)	1.39	52
LTH (cm)	1.58	34	1.53	33
MR (number)	1.24	49	(1.41)	(56)
VOL (cm)	5.19	64	5.53	68
QD (number)	0.66	35	0.72	38

[1]Assuming a 10 percent selection intensity, i = 1.755. Responses in parenthesis are for directly selected traits.

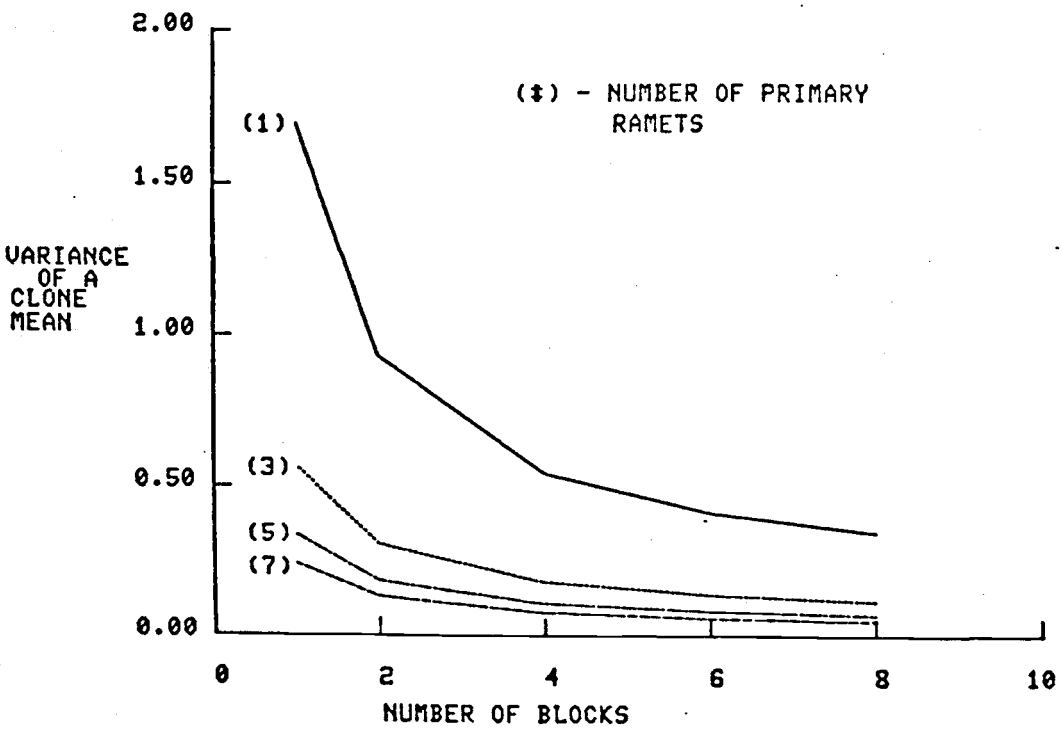

Figure 4. Variance of a clone mean for number of rooted cuttings per plot (RC) for different numbers of blocks and primary ramets per clone.

statistical efficiency and cost is needed. Based on variance data from this experiment, the greatest decrease in $V_{\overline{X}}$ occurs by increasing ramets from one to three (Figure 4). If three or more primary ramets are tested, a design using more than two blocks results in fairly minor reductions in $V_{\overline{X}}$.

Heritability (H) and predicted gain are also affected by changes in $V_{\overline{X}}$ due to design changes. As $V_{\overline{X}}$ decreases, the precision of estimating a clone mean increases, resulting in an increase in heritability toward a theoretical limit of one. Although heritability increases directly with decreasing $V_{\overline{X}}$, predicted genetic gain may not increase because larger heritabilities may be offset by a smaller phenotypic standard deviation. Referring to Figure 4, paired values of ramets (p) and blocks (b) of 3 and 4, 5 and 2, or 3 and 6 result in values of $V_{\overline{X}}$ of almost the same magnitude as in the original design ($p = 3$ and $b = 8$). Heritabilities calculated for these different designs are very similar and gains are almost identical (Table 13). The number of plots (pb) for the new designs is greatly reduced, leading to cost reductions, while the decrease in predicted gain appears to be minor.

Discussion

Results from both experiments indicate that C effects may play an important role in masking genotypic values of rooting traits in hemlock clones. The confounding influence of C effects can be minimized with some expense by using simple horticultural techniques, thereby improving selection for rooting ability. After one generation

Table 13. Estimated heritability and gain for number of rooted cuttings per plot (RC) using different methods of testing.

Number of primary ramets (p)	Number of blocks (b)	Heritability	Phenotypic standard deviation	Gain (%)	Number of plots (pb)
5	2	0.81	1.00	54	10
3	4	0.82	1.00	54	12
3	6	0.85	0.98	55	18
3	8	0.87	0.97	55	24

of selection among hemlock clones, improvement in proportion of rooted cuttings and quality of the root systems is predicted to be substantial. The information generated in this study is useful in determining whether hemlock can be economically employed in a clonal reforestation program (Chapter II).

If care is not taken to standardize the location of cuttings taken from tree crowns, this study shows a substantial proportion of the variance among clones may be due to C effects, i.e., the "M" effects of Burdon and Shelbourne (1974). Rooting characteristics depend partly on the origin of cuttings within the crown of the ortet. Of the two factors studied, vertical position within the tree crown is most influential although crown aspect may also have some effect. If the origin of cuttings is different among ortets, position-related effects among clones translate into C effects which lead to imprecise estimates of genotypic values of clones.

Although not directly tested, variation in age and physiological vigor of ortets may also cause differences in rooting ability, which if not accounted for in the analysis, could also mimic genetic differences among clones. In Experiment 1, ortet age may have had some influence on rooting ability. Clone 3 was both the oldest ortet (Table 2) and had the poorest rooting (Table 6). The product-moment correlation between the RC of these four clones and ortet age was -0.51. With only four ortets, however, the magnitude of this correlation may be an artifact of sampling. In an earlier study of 195 mature (19-60 years old) hemlock ortets, a product-moment correlation of -0.21 between RC and ortet age was obtained (Foster, Martin, and Caldwell,

1981). Therefore, ortet age can influence rooting ability of hemlock cuttings, but not necessarily to the extent indicated in Experiment 1.

The study suggests four techniques to increase precision in selecting genetically superior clones for rooting characteristics in hemlock: (1) standardize cutting collection procedures, (2) distribute plots of cuttings from a clone at random in the rooting chamber, (3) use secondary cloning, and (4) select clones for a highly heritable rooting trait. The first technique involves collecting cuttings from the same crown position in each ortet, preferably from the lower live crown. Secondly, distribute cuttings from each ortet in several locations within the rooting chamber. Rooting ability of cuttings varied among environments within the rooting chamber in both experiments (i.e., block effects were significant for all five traits). If all cuttings of a clone had been rooted in only one block in the rooting chamber, a clone mean might have reflected an interaction of the clonal genotype with a special environment within the block.

Heritability and phenotypic standard deviation are affected by blocking, which therefore indirectly influences genetic gain. Had clones not been blocked in the rooting chamber, clonal variation would have included clone and block x clone effects (i.e., $\hat{\sigma}^2_C + \hat{\sigma}^2_{BC}$). The phenotypic standard deviation would have included block effects ($\hat{\sigma}^2_B$) in addition to effects already in the error component. The bias introduced in heritability estimates would depend on the relative sizes of $\hat{\sigma}^2_{BC}$ and $\hat{\sigma}^2_B$ because $\hat{\sigma}^2_{BC}$ would be added to the numerator and $\hat{\sigma}^2_B$ would be added to the denominator. The phenotypic standard deviation would be larger by $\hat{\sigma}^2_B$.

The third technique involves secondary cloning (Figure 2), the process of rooting the cuttings (primary ramets) from the ortet, then taking cuttings (secondary ramets) from the rooted primary ramets. Secondary cloning can increase uniformity among secondary ramets if the primary ramets are treated uniformly. Cuttings taken from the primary ramets are usually more vigorous and uniform than cuttings taken from the ortet especially if primary ramets are hedged (T. D. Caldwell, Research Forestry Assistant, Crown Zellerbach Corp., Wilsonville, Oregon, personal communication). By hedging primary ramets, secondary cloning also produces large numbers of cuttings from which the most suitable can be chosen for rooting.

Secondary cloning provides an opportunity for partitioning the unique effects of primary ramets on rooting ability. A single ramet has the potential to influence the behavior of cuttings taken from it (Libby and Jund, 1962). By collecting several cuttings from each primary ramet and combining their rooting values across primary ramets, the effects of primary ramet differences are essentially eliminated. The process is analagous to accounting for environmental variation in a rooting chamber by blocking. Blocking by primary ramet in this study appears to have reduced the magnitude of variation due to C effects in Experiment 2 to about one-half its size, relative to genetic variation in Experiment 1. Direct evidence for this reduction of C effects due to secondary cloning is not available since different clones were used in the two studies.

The fourth technique is to select clones for a rooting trait with high heritability, providing such selection does not degrade other

traits. Selection for traits with high heritability is more efficient because the phenotype more closely reflects the genotype, thereby making genetically superior individuals easier to identify. For rooting traits in hemlock, this approach appears viable because genetic correlations among rooting traits were large and positive. By selecting for greater number of rooted cuttings per plot (RC), all other rooting traits should also be improved.

Biologically the five rooting traits are different (yet related) measures of a single process. For example, the longest main root (LTH) often comprised a large proportion of the sum of main roots (VOL), leading to the high genetic correlation (0.93) between LTH and VOL. Similarly as the number of main roots (MR) on a cutting increased, the sum of their lengths (VOL) increased, and the roots tended to be distributed more completely around the base of the cutting (QD). Clones which rooted well (high RC) also had good root systems, which led to high genetic correlations between RC and the other traits (Table 11). Although not addressed in this study, time to rooting may be an important factor. Easy-rooting clones may have rooted earlier and subsequently had more time to develop their root systems prior to measurement.

Using the various horticultural techniques to increase precision in clonal selection increases the cost of rooting. For the techniques to be worthwhile, added benefits must outweigh added costs. For example, placing the cuttings from each ortet in several, scattered blocks in the rooting chamber requires both added time and labor. Cuttings must be sorted, carefully labeled, and kept track of

throughout the setting, rooting, and lifting process. Secondary cloning also has additional cost associated with it; the cost of maintaining and hedging the primary ramets as well as the longer time before achieving the predicted genetic gain in rooting ability. If gain for rooting ability is expressed on a per year basis, then the secondary cloning process adds time to the cycle length, reducing predicted gain per unit time. Although predicted gain per unit time may be greater without secondary cloning and the costs associated with secondary cloning are significant, the projected increase in realized gain in rooting ability after secondary cloning may offset these costs because of more precise identification of superior clones.

Large genetic gains appear to be possible by selecting for improved rooting characteristics. Estimates of the two factors most important in predicted gain, heritability and variation among clones, were large for all measured traits. The large heritability values (0.87 to 0.92) indicate that these traits are under strong genetic control and that the rooting design has been very effective.

Previously published estimates of heritability for rooting traits in several tree species, including hemlock, are not directly comparable to this study because they were calculated on an individual-cutting basis (Wilcox and Farmer, 1968; Fancher and Tauer, 1981) or a plot basis (Sorensen and Campbell, 1980; Foster, Martin, and Caldwell, 1981). In this study, heritabilities were calculated on a clone mean basis because unless clones are to be selected on the performance of a single rooted cutting or a single plot, the appropriate heritability estimate should be based on clone means. After converting the

heritability estimates for hemlock given in Foster, Martin, and Caldwell (1981) to a clone mean basis (assuming no missing plots), the values for RC (0.86 - mature ortets and 0.82 - juvenile ortets) and MR (0.82 - mature population, and 0.86 - juvenile population) are very close to those in this study (RC = 0.87, MR = 0.92).

Variation among clones for the rooting traits was large, contributing to the substantial genetic gain predicted for each trait. The range in clone means (Table 10) was considerable; and in addition, the coefficient of variation of clone means (Table 10) averaged 35 percent. The large predicted gains (Table 10) seem reasonable since genetic gain depends heavily on both heritability and variation among clone means.

Both the heritabilities and the variation among clones, however, may still be biased upward by C effects that could not be removed in Experiment 2 (e.g., tree age influence on rooting ability). Therefore, predicted gains probably are overestimates. Without secondary cloning, the overestimates may have been even larger. Using the biased heritability estimates presented previously, predicted percentage gains were 64 (RC), 51 (LTH), 57 (MR), 80 (VOL), and 39 (QD). Compared to the gains predicted after secondary cloning (Table 10), omitting secondary cloning causes an average upward bias of about 3.6 percent.

Both the percentage of rooted cuttings and quality of root systems could be rapidly improved in hemlock through clonal selection. Increasing average rooting ability would have a large economic impact on clonal reforestation programs. With gains in rooting, sizes of

cutting orchards, rooting facilities and aftercare facilities could be reduced. The time between lifting of rooted cuttings and outplanting in the field could be shortened due to the better quality root system at lifting. Finally, improved quality of root systems may have a long term effect on enhancing the subsequent field growth of rooted cuttings. Foster (1978) and Foster, Bridgwater, and McKeand (1981) suggested a combined selection scheme for rooting ability and the subsequent growth rate of rooted cuttings.

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CHAPTER IV. CLONAL SELECTION IN WESTERN HEMLOCK COMBINING ROOTING TRAITS WITH TOTAL HEIGHT GROWTH

Introduction

Inheritance accounts for a substantial part of the variation in both rooting ability (Sorensen and Campbell, 1980; Foster, Martin, and Caldwell, 1981) and growth rate (Foster and Lester, 1983) in western hemlock. Thus, both should be susceptible to improvement through selection of superior clones. When selecting for more than one trait, however, three somewhat different procedures can be used: tandem selection, independent culling level, or index selection (Hazel and Lush, 1942). Based on theoretical considerations (Hazel, 1943) and practical demonstration (Hazel and Lush, 1942), index selection is considered to be the most efficient of the three. This chapter presents results of an experiment in which a selection index is used to evaluate combined selection for rooting characteristics and juvenile height in western hemlock.

Wilcox and Farmer (1968) and Ying and Bagley (1974) evaluated the association between rooting ability and shoot growth for cottonwood clones. While Wilcox and Farmer (1968) found no significant association between these traits, Ying and Bagley (1974) found they were positively correlated ($r = 0.17$) for first year height growth.

Even though the potential benefits of using selection indices in forest tree improvement are large (Stonecypher, 1970), forest geneticists have been slow to make use of them. This reluctance has been due to several problems with their application in forestry (Arbez *et*

al., 1974). These problems include: (1) economic weights are beneficial for selection indices, yet they are difficult to determine in forestry because of changing market conditions and (2) index construction depends upon accurate estimates of genetic and phenotypic variances and covariances, which can only be obtained from large and precise studies. Despite these problems, forest geneticists are beginning to employ index selection more and more frequently while attempting to find solutions to the aforementioned problems (personal communication, Dr. F.E. Bridgwater, Forest Geneticist, U.S. Forest Service, Raleigh, N.C.).

Index selection has been utilized for multiple trait selection of cottonwood clones (Bridgwater, 1972). Height, diameter, specific gravity, volume, and dry weight were included in Bridgwater's index as traits for improvement. He dealt with the problem of inadequate economic information by conducting a sensitivity analysis of economic weights.

The objectives of this current study were: (1) provide information to the network analysis (Chapter II) of research and development for clonal reforestation with hemlock, (2) to determine genetic and phenotypic associations between rooting traits and juvenile height growth (first year) of hemlock clones, (3) to compare genetic gains for single-trait selection on juvenile height with the accompanying indirect response for rooting traits, as opposed to combined selection of both types of traits utilizing index selection, and (4) to use the information from Chapters III and IV to recommend a selection procedure for juvenile growth and rooting ability.

Aspects of several research activities listed in the network diagram in Chapter II are included in this study. These activities are: (1) 200;220 - study relationship between rooting ability and growth of cuttings, (2) 400;420 - determine heritability, genetic correlation, and gain for growth traits, and (3) 400;430 - assess magnitude of C effects on growth traits. Activity 200;220, however, is only partially addressed. While ten years of growth data are required to complete this activity, only one year of growth was measured in this study.

Materials and Methods

The clones used in this study were a subset of those in Experiment 2 (Chapter III). Of the original 60 clones in the rooting experiment, 30 were chosen from the clones which produced enough rooted cuttings to meet the study design requirements. The rooting environment was described in Chapter III.

The same five rooting traits investigated in Experiment 2 were again included in this study. These traits were: number of rooted cuttings per five cutting plot (RC), length of the longest main root on each rooted cutting (LTH), number of main roots (roots beginning at the cutting base) per rooted cutting (MR), sum of the lengths of all main roots per rooted cutting (VOL), and number of quadrants (of the cutting base) from which main roots arise per rooted cutting (QD). Since a cutting must have rooted to be used in this study, there were no zero values for any of the traits.

Following lifting and evaluation of rooting traits in July, 1981, rooted cuttings from 30 clones in Experiment 2 were potted into 352.6 ml book planters. The potting medium consisted of equal parts of sand, shredded peat moss, and perlite. The medium was steam sterilized before use. The potted, rooted cuttings were than placed in a greenhouse and maintained at temperatures between 10°C and 20°C (using heating as needed) for the next 6 months. The cuttings received a normal (avoiding unnatural water stress) watering regime during this period.

From the greenhouse, the rooted cuttings were moved (one year after rooting was initiated) to two growth chambers (Model M-1148, Environmental Growth Chamber Co.) and subjected to a growing regime which had proven successful in previous experiments with hemlock seedlings in these chambers (personal communication, Dr. Bruce Rottink, Research Forester, Crown Zellerbach Corp., Wilsonville, Oregon). During the initial four week period in the chambers, short daylengths (eight hr. light) were used along with constant temperature (5°C) and relative humidity (60-70 percent) (Table 14), and the rooted cuttings were watered on a normal schedule. The intent of this regime was to insure that the buds of the cuttings had fulfilled their cold requirement. After an eight-day transition period of increasingly warmer temperatures and increasing day length, the growing environment was held essentially at a constant regime for the remainder of the growing period (three months) (Table 14).

Lighting was a combination of G. E. (General Electric Co.) high intensity discharged lamps. Each of the three fixtures per chamber

Table 14. Environmental conditions in the hemlock rooted cutting growth experiment.

Date	Temperature (°C)	Relative humidity (percent)	Duration of light intensity[1] (hrs:min)							
			0[2]	1	2	3	2	1	0	1
1/11/82 - 2/11/82	5	60-70	16:00	1:45	2:00	0:30	2:00	1:45		
2/12/82	7	50	15:20	1:55	2:10	0:30	2:10	1:55		
2/13/82	9	50	14:40	2:05	2:20	0:30	2:20	2:05		
2/14/82	11	55	14:00	2:15	2:30	0:30	2:30	2:15		
2/15/82	13	60	13:30	2:30	2:30	0:30	2:30	2:30		
2/16/82	15	65	13:00	2:30	2:45	0:30	2:45	2:30		
2/17/82	17	65	12:30	2:45	2:45	0:30	2:45	2:45		
2/18/82	19	65	12:00	2:45	3:00	0:30	3:00	2:45		
2/19/82 - 4/11/82	21	65	5:45	2:45	3:00	0:30	3:00	2:45	5:45	0:30
4/12/82 - 5/28/82	21	65	5:45	2:45	3:00	1:00	2:30	2:45	5:45	0:30

[1]From 2/19/82 - 5/28/82, the 11 1/2 hour night was broken in half by one half hour of light.

[2]Light intensities: 0 - dark, 1 - 150 $\mu Em^{-2}sec^{-1}$, 2 - 290 $\mu Em^{-2}sec^{-1}$, 3 - 430 $\mu Em^{-2}sec^{-1}$.

contained two types of bulbs: (1) a G. E. High Intensity Discharge "R Multi-Vapor Lamp" 400 watt, and (2) a G. E. High Intensity Discharge Lucalox High Pressure Sodium Lamp bulb. These bulbs could be adjusted to three intensities (150, 290, and 430 $\mu Em^{-2}sec^{-1}$) referred to, respectively, as light intensities 1, 2, and 3. Instead of a constant light intensity during the day, the light intensity varied to more closely simulate natural lighting patterns with increasing intensities in the morning reaching a peak at noon then decreasing.

The trees were watered once a week, either with de-ionized water or fertilized with a soil drench, on approximately an alternating basis from February 25, 1982 until the end of the study. The fertilizer was a 1:1:1 ratio of nitrogen, phosphorus, and potassium applied at 150 ppm in de-ionized water plus 30.1 gm of Sequestrene iron and 0.75 gm of Peter's Special S.T.E.M. (Soluble Trace Elements Mix) micronutrients in 208.5 1 of de-ionized water.

On May 27 and 28, 1982, total height (initial cutting height (CUTLTH) plus height growth) of each rooted cutting was measured to the nearest half cm. The typically drooping terminals of hemlock were straightened for measurement. Survival of rooted cuttings at the end of the growing period was 99.5 percent.

Two different analyses were used in the growth experiment. The experimental design consisted of 2 growth chambers and 5 blocks per chamber. Each block contained 30 clones and 3 primary ramets per clone (see Chapter III for an explanation of primary ramets) with each primary ramet represented by a plot of 2 rooted cuttings. The resulting analysis permitted partitioning juvenile height (HT)

variation into several environmental components and a genetic component. The form of the analysis of variance is presented in Table 15. Growth chambers were fixed effects while all other effects were random. The analysis was based on plot means. Some missing plots occurred when the study was installed due to a shortage of rooted cuttings for some clones. In the data analysis, missing plots were left blank, and a least squares analysis was employed since it can accommodate missing values. Since the analysis was unbalanced, coefficients of variance and covariance components (Table 15) were adjusted (Searle, 1971).

Because an estimate of covariance between traits was needed to calculate genetic correlations, evaluation of rooting traits and total height (HT) had to be based on the same arrangement of experimental units. This required a second analysis. That is, Experiment 2 (Chapter III) included five cuttings per plot and eight blocks, while the growth experiment included two cuttings in a plot and ten blocks (five blocks in each of 2 chambers). Also, in Experiment 2, some blocks had five rooted cuttings while some had none. This required a new randomization of rooted cuttings (within plots and blocks) from Experiment 2 to the growth experiment. In the second model, a nested design is assumed with 30 clones, 3 primary ramets per clone, and 10 plots per ramet with completely random assignment of two-tree plots (i.e., no blocking). The form of the analysis of variance (or covariance) is presented in Table 16.

Because rooting traits and height growth were measured in different experiments with different designs, significant environmental

Table 15. Form of the analysis of variance for total height (HT) of hemlock clones grown in a controlled environment.

Source of variation	Degrees of freedom	Expected mean squares[1]
Growth chambers (H)	1	$\sigma^2 + b\sigma^2_{HP(C)} + bp\sigma^2_{HC} + cp\sigma^2_{B(H)} + bcp\theta^2_H$
Blocks (B)/H	8	$\sigma^2 + cp\sigma^2_{B(H)}$
Clones (C)	29	$\sigma^2 + hb\sigma^2_{P(C)} + hbp\sigma^2_C$
Primary ramets (P)/C	60	$\sigma^2 + hb\sigma^2_{P(C)}$
H x C	29	$\sigma^2 + b\sigma^2_{HP(C)} + bp\sigma^2_{HC}$
H x P/C	60	$\sigma^2 + b\sigma^2_{HP(C)}$
Error	712	σ^2

[1] h, b, c, and p are adjusted values of the number of growth chambers, blocks, clones, and primary ramets.

θ^2_H = effects due to growth chamber differences.

$\sigma^2_{B(H)}$ = variance among blocks within growth chambers.

σ^2_C = variance among clones.

$\sigma^2_{P(C)}$ = variance among primary ramets within clones.

σ^2_{HC} = variance due to interaction of growth chambers and clones.

$\sigma^2_{HP(C)}$ = variance due to interaction of growth chambers and primary ramets within clones.

Table 16. Form of the analysis of variance (and covariance) used for estimating components of variance (and covariance) for total height (HT) and rooting traits (RC, LTH, VOL, MR, and QD) of hemlock clones.

Source of variation	Degrees of freedom	Expected mean squares[1]
Clones (C)	29	$\sigma^2 + n\sigma^2_{P(C)} + np\sigma^2_C$
Primary ramets (P)/C	60	$\sigma^2 + n\sigma^2_{P(C)}$
Error	810	σ^2

[1]n was the number of plots per primary ramet and p was the number of primary ramets per clone.

σ^2_C = variance among clones.

$\sigma^2_{P(C)}$ = variance among primary ramets within clones.

σ^2 = error variance.

variation associated with blocking (i.e., blocks in rooting chambers and growth chamber effects in the growth study) had to be accounted for. I adjusted the rooting data (from Experiment 2, Chapter III) on each rooted cutting to alleviate block differences and the height data on the same rooted cuttings to alleviate growth chamber differences. After an additive arithmetic adjustment of data on individual cuttings, plot means were calculated. Since the analysis was based on plot means of the two-tree plots, each primary ramet had ten data points, one from each of the previous five blocks in each chamber.

Variance and covariance components for the five rooting traits (RC, LTH, VOL, MR, and QD) and total height (HT), were derived from the second analysis, by equating mean squares (or mean cross products) to expected mean squares (or mean cross products) and solving equations. Broad-sense heritabilities on a clone mean basis were calculated for all traits as follows:

$$H = \frac{\hat{\sigma}^2_C}{\hat{\sigma}^2_C + \frac{\hat{\sigma}^2_{P(C)}}{p} + \frac{\hat{\sigma}^2}{np}}$$

Genetic and phenotypic correlations among the six traits were also estimated using the technique of Johnson, Robinson, and Comstock (1955).

To assess potential gains that may be made by combining selection for HT with selection for rooting traits, selection indices were calculated. Indices, direct gains, and indirect gains were calculated using techniques described by Van Vleck (1981).

Selection indices normally require estimates of the net economic value of each trait. Unfortunately none of the rooting traits measured in this study or first year height of hemlock have been assessed for their economic value. A sensitivity technique (Bridgwater, 1972) was therefore used for assigning relative economic weights to the traits. Since increased yield is a main objective of most tree improvement programs and juvenile height is an early indication of yield, the relative economic weight of 1.0 was assigned to HT, and weights of 1.0, 0.5, and 0.0 were assigned to one rooting trait in separate calculations. Ideally, the impact of root-shoot ratios on subsequent tree growth could be used to assign relative economic values to rooted cutting height and rooting traits, but this information is not available for hemlock.

Results

Although height growth was the only trait measured in the growth study, rooting data were available from Experiment 2 (Chapter III) for each of the rooted cuttings. Because 20 cuttings per ramet were needed for the design in the growth experiment, clones which rooted poorly tended not to be used in the growth study. This truncation of the original population of 60 clones resulted in the 30 clone sample having higher mean values for each of the rooting traits compared to the original population (Table 17). In effect, selection pressure was inadvertently applied to the original population in order to obtain clones with enough rooted cuttings for the growth experiment.

Table 17. Mean values (standard errors) for five rooting traits and total height in two samples of hemlock clones.

Trait	Samples[1]	
	60 clone	30 clone
HT (cm)	--	20.13 (.15)
RC (number)	2.67 (.04)	3.92 (.03)
MR (number)	2.50 (.04)	3.13 (.05)
QD (number)	1.90 (.02)	2.23 (.03)
LTH (cm)	4.65 (.07)	5.79 (.08)
VOL (cm)	8.12 (.17)	11.33 (.22)

[1]The 30 clone sample was a subset of the 60 clone sample.

After annual growth was completed in the growth chambers, height variations due to differences between growth chambers, among clones, and among primary ramets within clones were all found to be significant (Table 18). Although the two growth chambers were set for the same environmental conditions, the rooted cuttings in one chamber averaged 1.35 cm taller than those in the other chamber. The plot-mean height of cuttings was 20.13 cm with a range of 7.25-34.75 cm (Table 19). The coefficient of variation for HT was 12 percent, about one-third the size of the coefficients of variation for the rooting traits (Chapter III). Clonal variance in HT, which provided an estimate of total genetic variance, accounted for 21 percent of the total variation in the experiment, and variance due to differences among primary ramets within clones (C effects variance) accounted for eight percent (Table 18). Thus, for total height, variation due to C effects was approximately one-third the size of the total genetic variation.

The second analysis (Table 20) made it possible to obtain estimates of variance and covariance components from the same data set. Components were then used to estimate genetic parameters. Broad-sense heritability of HT was 0.81 (Table 19). With C effects included, H = 0.92. The heritabilities estimated for the rooting traits from this sample of 30 clones were similar but consistently lower than those obtained using data from all 60 clones [i.e., RC (0.66 vs. 0.87), MR (0.87 vs. 0.92), QD (0.86 vs. 0.89), LTH (0.87 vs. 0.87) and VOL (0.86 vs. 0.90)].

Table 18. Analysis of variance for total height of hemlock clones using original model.

Source of variation	Mean squares	Variance component[2]	Estimate[3]	Percent of total
Growth chambers (H)	400.35**[1]	θ^2_H	0.89	4
Blocks (B)/H	18.91^{NS}	$\sigma^2_{B(H)}$	0.06 (0.10)	1
Clones (C)	158.41**	σ^2_C	4.45 (1.40)	21
Primary ramets (P)/C	30.15**	$\sigma^2_{P(C)}$	1.75 (0.57)	8
H x C	9.09^{NS}	σ^2_{HC}	0.00 (0.25)	0
H x P/C	15.48^{NS}	$\sigma^2_{HP(C)}$	0.45 (0.60)	2
Error[4]	13.31	σ^2	13.31 (0.72)	64

[1]A synthetic F test, after the technique of Cochran (1951), was used to test for differences among growth chambers.

[2]Adjusted values of the coefficients of variance components were: h = 2, b = 4.8, c = 28.8, and p = 3 (refer to Table 15 for symbols).

[3]Standard error (Namkoong, 1979) in parenthesis.

[4]Actual error degrees of freedom was 678 due to missing plots.

** Significant at 1 percent level.

NS Not significant at the 5 percent level.

Table 19. Parameter estimates for first-year height (HT) in a population of hemlock clones.

Parameter	Value
Mean ($\overline{X}$)	20.13 cm
Heritability (H)	0.81 (.06)[1]
Phenotypic standard deviation ($\hat{\sigma}_X$)	2.35 cm
Coefficient of variation ($\frac{\hat{\sigma}_X}{\overline{X}}$)	12 percent
Range of plot means	7.25-34.75 cm

[1]Standard error of heritability (Namkoong, 1979).

Table 20. Analysis of variance for six traits of hemlock clones using the second model.

Source of variation	Mean squares[2]					
	HT	RC	MR	QD	LTH	VOL
Clones (C)	158.36**	6.12**	24.25**	5.72**	51.23**	530.00**
Primary ramets (P)/C	30.10**	2.09*	3.11*	0.78**	6.88*	72.54**
Error[1]	13.37	0.38	1.35	0.47	3.59	20.77

[1]Actual error degrees of freedom was 810 due to missing plots.

[2]Adjusted values of the coefficients of variance components were: n = 9.6 and p = 3 (refer to Table 16 for symbols).

* Significant at 5 percent level.

** Significant at 1 percent level.

Juvenile height is genetically associated with desirable attributes of rooting genetic correlations were moderately large (Table 21). The genetic correlations of HT with the five rooting traits ranged from 0.37 to 0.59, and phenotypic correlations ranged from 0.35 to 0.53. As occurred in the 60 clone sample (Chapter III), the rooting traits were highly and positively correlated both genetically and phenotypically.

Given the large genetic correlations among the five rooting traits, only one of the traits, VOL, was chosen for use in a selection index with HT. VOL was chosen over the other rooting traits for two reasons: 1. except for QD, VOL had the largest genetic correlation with HT and 2. the measurement of VOL is much less subjective than for QD. The gains from this index were compared to gains from selection for HT alone (Table 22).

With a positive, genetic correlation between HT and VOL, two selection strategies are feasible, depending on the value of gain in VOL relative to HT. A positive economic weight could be applied to both traits or VOL could be used as an aid for selecting clones for HT even though 0.0 economic weight is applied to VOL (Van Vleck, 1981). Expected gains in HT were relatively insensitive to changing relative economic weights for VOL (Table 22). The greatest gain in HT occurred either when VOL was given an economic weight of 0.0 and was used as an aid to select clones for HT, or when HT was selected alone.

Table 21. Phenotypic (above diagonal) and genetic (below diagonal) correlations and their standard errors (in parentheses) among five rooting traits and height of hemlock cuttings.

Trait	Trait					
	RC	MR	LTH	VOL	QD	HT
RC		0.71 (0.09)	0.51 (0.13)	0.73 (0.09)	0.71 (0.09)	0.35 (0.16)
MR	0.91 (0.12)		0.47 (0.14)	0.85 (0.05)	0.95 (0.02)	0.46 (0.14)
LTH	0.63 (0.17)	0.48 (0.16)		0.83 (0.05)	0.49 (0.14)	0.50 (0.14)
VOL	0.95 (0.11)	0.87 (0.06)	0.84 (0.06)		0.81 (0.06)	0.52 (0.13)
QD	0.87 (0.11)	0.98 (0.02)	0.53 (0.16)	0.87 (0.07)		0.53 (0.13)
HT	0.37 (0.22)	0.49 (0.17)	0.50 (0.16)	0.53 (0.16)	0.59 (0.15)	

All phenotypic correlations are significant at the 1 percent level.

Table 22. Genetic gains in measurement units (and percent of mean) for hemlock clones from different selection strategies emphasizing HT and VOL.

Relative economic weights			Gain					
			HT	VOL	LTH	QD	MR	RC
HT	VOL	Index	(cm)			(number)		
1.0	1.0	I = 0.84 HT + 0.88 VOL	1.67(8)	3.84(34)	1.04(18)	0.36(16)	0.72(23)	0.33(8)
1.0	0.5	I = 0.81 HT + 0.46 VOL	1.85(9)	3.59(32)	0.98(17)	0.35(16)	0.68(22)	0.30(8)
1.0	0.0	I = 0.78 HT + 0.037 VOL	2.09(10)	2.31(20)	0.62(11)	0.32(14)	0.45(14)	0.17(4)
1.0		I = 0.81 HT	2.08(10)	2.06(18)	0.61(11)	0.24(11)	0.41(13)	0.13(3)

[1]Assume a selection intensity of 10 out of 30 clones or i = 1.097.

Discussion

Significant clonal variability and large heritability creates an opportunity for achieving genetic gain in juvenile height (HT) of hemlock through selecting the superior clones. The results of this study suggest two ways for making clonal selection for HT more effective. The first step is to design clonal tests to minimize C effects for HT; the second step is to use combined selection for growth and rooting traits. These findings add to the information from experiments in Chapter III to answer research and development questions presented in Chapter II.

Although the heritability for HT was within the range of the values for the rooting traits, the coefficient of variation for HT (Table 19) was only about one-third the size of those for rooting traits (Table 10, Chapter III). This resulted in smaller predicted gain from direct selection for HT than for rooting traits. The selection pressure placed on the choice of 30 clones from the original 60 clone population undoubtedly reduced the variability of HT, resulting in lower predicted gain.

Secondary cloning should precede clonal testing for height otherwise variation due to C effects will bias predicted genotypic values of clones as well as estimated genetic parameters. If, for example, realized heritability was actually ten percent less than predicted (i.e., H = 0.73), due to the masking of genotypic value by C effects, genetic gain would decline from a predicted value of 10 percent (with secondary cloning) (Table 22) to a realized value of 9

percent (gain = 1.88 cm in HT). Given the size of C effects (one-third as large as clonal variation) for HT, the actual decrease in realized heritability and selection intensity without secondary cloning may be even greater than ten percent. This is in contrast to the predicted gain of 12 percent in HT using heritability (0.92) estimated without secondary cloning.

C effects variation for HT arose despite efforts to minimize it through hedging and uniform growing conditions for the primary ramets. This non-genetic variation could have originated in the original condition of the cutting itself such as cutting size (i.e., product-moment correlation between cutting length and HT was 0.13), age, or vigor or indirectly through C effects passed on from rooting traits to HT. C effects were large even after hedging and may have been even larger without it (i.e., rooting traits in Chapter III).

Once secondary cloning has reduced variation due to C effects, selection will be more effective for improving HT as well as rooting traits. The association between the rooting traits and HT was positive and significant, in contrast to the results of Wilcox and Farmer (1968) who found no correlation between any root measurement and subsequent early height growth. The time and conditions of measurement may account for the inconsistency. Ying and Bagley (1974), using the same clones, found that the correlation between number of roots per cutting and cutting height growth decreased with increasing age of the plantation (i.e., from 0.17 at age one to -0.04 at age seven). Ying and Bagley's results suggest that early height growth is related to rooting ability (especially for clones which root poorly) through a

limiting process; size of the root system may limit shoot growth through physiological processes. After the root system has had time to develop fully in the field, the limiting association declines and finally disappears. The early, positive correlation, however, may mean that clones which root poorly may grow slowly in the year or two after outplanting and may not survive well in the harsher environments of commercial plantations (personal communication, T. D. Caldwell, Research Forester Assistant, Crown Zellerbach Corp., Wilsonville, Oregon).

Disregarding, for the moment, the benefits of an improved root system, selection for rooting traits theoretically could be used to improve selection for juvenile height--because of the strong positive correlation among HT and rooting traits. In fact when VOL with zero economic weight was included in the selection index, gain in height (2.09 cm) was essentially the same as selection for height alone (2.08 cm). With larger economic weights for VOL, gain in height is decreased. In the absence of actual economic weights, the selection index was tested for sensitivity to changing weights for VOL when the weight on HT remained constant. When the weight for VOL increased from 0.0 to 0.5, there was a substantial increase in gains (Table 22) for VOL (20 percent to 32 percent) with a minor decrease in gains for HT (10 percent to 9 percent). In addition to larger gains in VOL, gains in LTH, QD, MR, and RC also increased substantially. Little additional improvement in gains for VOL resulted from further increases in its economic weight.

Improving initial rooting ability on rooted cuttings has several economic benefits. These include: (1) fewer primary ramets will be needed to provide the necessary number of cuttings, thus saving maintenance and development costs for the primary ramets, (2) smaller rooting facilities and therefore less labor and supplies will be needed to supply the necessary rooted cuttings, (3) costs associated with aftercare, following lifting of cuttings from the rooting bench, may be less if the rooted cuttings are ready for field planting in a shorter period of time, and (4) if survival of field-planted cuttings is higher, then fewer rooted cuttings will be needed to achieve a desired stocking level and associated planting costs will be reduced. Consequently, improving rooting ability in a population of hemlock clones may have substantial economic benefits throughout the entire propagation and stand establishment process.

There is some evidence that the positive correlation between rooting ability and height growth may diminish with increasing stand age (Ying and Bagley, 1974). This suggests that other experiments (i.e., relate rooting ability to older growth traits in field experiments) are needed before deciding on a clonal selection system for commercial use. Results of this experiment provide several guidelines. Use secondary cloning to provide the cuttings for clonal testing, paying close attention to uniformity of the primary ramets (Chapter III). Employ an efficient randomized complete block layout in the rooting chamber (i.e., 3 primary ramets per clone and 4 blocks, 5 primary ramets per clone and 2 blocks, or 3 primary ramets per clone and 6 blocks, Chapter III). Record RC and VOL for the cuttings in the

rooting chamber, then pot the rooted cuttings for field planting. Cull as few clones as possible at this stage because the relationship between rotation-length, growth performance and rooting ability of clones is unknown. Out-plant the rooted cuttings in a replicated field design, and measure survival and growth performance of the clones over time. In all the above steps, careful records should be kept on costs associated with hedging, secondary cloning, blocking, and rooting. This information is needed to develop appropriate economic weights, along with quantitative data to support the benefits of improving initial rooting ability. Data from field tests carried to 1/3 or 1/2 rotation age are needed to confirm the value of using juvenile HT and VOL in a selection index for improving yield at harvest. Predicted gains from the experiment reported in Chapter III apply only to first year height since the relationships are unknown between (1) rooting and older tree height and survival and (2) younger and older tree heights or volumes. Research on these relationships is needed.

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