Breeding Blister Rust Resistant Western White Pine

V. Estimates of Heritability, Combining Ability, and Genetic Advance Based on Tester Matings

By R. T. Bingham1, R. J. Olson2, W. A. Becker3, and M. A. Marsden4

(Received for publication August 30, 1967)

Throughout the commercial range of Pinus monticola Douglas and over extensive high-rust-hazard areas within the ranges of P. strobus L. and P. lambertiana Dougl., severe damage has occurred from blister rust disease (causal pathogen Cronartium ribicola J. C. Fisch. ex. Rabenh.). This damage is increasing, and North American foresters are looking more and more toward resistant stocks as the most promising means of assuring continued production of these important white pines.

In response to increasing need for resistant planting stocks, breeding programs aimed at mass production of partially resistant types are already underway—in Ontario and the lake states with P. strobus (Heimburger 1958, Patton and Riker 1966, Rudolph 1968), in the Pacific Northwest with P. monticola (Howard 1959, Bingham 1964), and in the California-Oregon region with P. lambertiana (Howard 1959, Oford 1961).

But the early-generation, partially resistant stocks now in sight are only the first, interim products of long-range breeding programs. White pine breeders are looking forward to advanced-generation stocks that are more resistant, buffered against a variety of rust races, faster growing, and otherwise improved. Since breeding programs require large expenditures of time and money, there is a growing need for precise information that will permit the breeder to make sound estimates of genetic variances, heritability, and genetic gain, using early-generation material and considering alternative breeding schemes.

This paper seeks to fill part of that need with respect to blister rust resistance, presenting estimates of (a) nonadditive and additive genetic variance, (b) general and specific combining ability, (c) narrow-sense heritability, and (d) genetic gain under alternative breeding schemes.

Literature Review

The literature on variation and inheritance of resistance to Cronartium (or Peridermium) rusts had an early beginning (Eriksson 1896, Liro 1907, Hartley 1927) and has since expanded to include several hundred references. No attempt will be made here to cover this fairly extensive literature. Only that part most pertinent to this study, that is, the literature concerned with mode of inheritance and heritability of tree rust resistance, will be discussed.

Presently, evidence on either the numbers or kind of genes controlling tree resistance to rust fungi is almost entirely lacking. Both Heimburger (1962) and Bingham (1964 and 1966) have speculated that blister rust resistance in P. strobus and P. monticola is controlled by polygenes. Muhle-Larsen (1964), however, has demonstrated that dominant genes control resistance to a Melampsora rust in certain Populus deltoides Bar. selections.

Somewhat more information is available concerning heritability of resistance to tree rusts (Hattaner 1964), but even on this subject, as far as can be determined, the literature is limited to the five references summarized below.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Host</th>
<th>Plant Material</th>
<th>Heritability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. ribicola</td>
<td>P. monticola</td>
<td>Full-sib families</td>
<td>.87 .69</td>
<td>Bingham et al., 1960</td>
</tr>
<tr>
<td>&quot;Birch rust&quot;</td>
<td>Betula japonica &amp; B. verrucosa</td>
<td>Full-sib families</td>
<td>.59 .52</td>
<td>Stern, 1962</td>
</tr>
<tr>
<td>C. fusiforme</td>
<td>P. elliottii</td>
<td>Half-sib families</td>
<td>— .33</td>
<td>Goddard and Arnold, 1966</td>
</tr>
<tr>
<td>Melampsora medusae</td>
<td>Populus deltoides</td>
<td>Clonal lines</td>
<td>.66—.88 —</td>
<td>Jokela, 1966</td>
</tr>
<tr>
<td>THUM.</td>
<td></td>
<td>Half-sib families</td>
<td>— .39—.66</td>
<td></td>
</tr>
</tbody>
</table>

Materials and Methods

In 1950 the Intermountain Forest and Range Experiment Station and Region One of the U.S. Forest Service began work attempting to develop P. monticola resistant to the white pine blister rust disease. Past progress has been

---

1) Principal Plant Geneticist and Statistician, respectively, U. S. Dept. Agr., Forest Service, Intermountain Forest and Range Experiment Station, Forestry Sciences Laboratory, Moscow, Idaho.
2) Public Health Service Predoctoral Fellow and Associate Professor of Genetics, respectively, Washington State University, Pullman, Washington.
3) Assistant Statistician, respectively.
reported (Bingham 1964, 1966; Bingham et al. 1983, 1986). During the 16 years through 1965, more than 400 rust-free white pines have been located in about 25 different rust-decimated natural stands in northern Idaho and adjacent states. These trees were candidates for use in production of resistant planting stock and were tested to determine their ability to transmit resistance to their seedling offspring.

In the earliest tests, as a result of the many missing crosses of the mating scheme, analysis was difficult and estimates of heritability and gain were not too reliable. In later tests originating in 1960 through 1963, however, a balanced tester mating scheme was used. Four tester trees, chosen on the basis of high resistance of their progenies, were mated with each new candidate. In addition, controls consisting of seedlings grown from open-pollinated seed of ordinary, susceptible trees of the base (or wild) population were entered in the tests.

This paper deals with three progeny tests (or experiments) carried out in a blister rust resistance test nursery at Moscow, Idaho, in the autumn of 1960, 1962, and 1963. In most matings tester trees were pollen (paternal) parents and candidates seed (maternal) parents, but in several these roles were reversed. However, because unpublished data from this program indicated a lack of reciprocal and maternal effects, candidates and testers were analyzed without regard to their role as pollen or seed parent.

In the 1960 test, each of 52 candidates was mated to 4 testers for a total of 208 crosses; in the 1962 test there were 36 candidates and 144 crosses. Tester trees were the same in both these years. In the 1963 test, 40 candidates were mated to a different group of 4 testers, resulting in 160 crosses. There were 15 different control progenies: 10 were used in the 1960 test, 6 in the 1962 test, and 8 in the 1963 test. One control progeny was used in all three tests, and others were common to two tests. The location of the various candidates, testers, and controls is shown in Figure 1.

**Figure 2.** — Two steps in test planting: A. Presowing the 10-block-long strips of paper toweling. Strips were stenciled to position seed spots and block divisions (left), and methyl cellulose mucilage was applied to the 100 seed spots (right) prior to sowing seeds in the mucilage and setting the strips aside to dry (background); B. the 9- by 3-inch plot positioned randomly within a block (replicate), showing the 18 two-year-old seedlings 1 year after artificial inoculation with the blister rust fungus.

**Experimental Design and Inoculation Procedures**

In each test, seeds were presown on paper toweling (Figure 2 A), then planted in the nursery in a design consisting of 10 randomized complete blocks, each block being a replicate and containing one 16-seed plot of each progeny (Figure 2 B). In anticipation of erratic germination, up to three seeds were sown in each planting spot.

Even though precautions were taken to secure full stocking of plots, the average numbers of seedlings per plot (m) dropped to 13.6 in the 1960 test, 11.8 in 1962, and 12.0 in 1963 — that is, to 74 to 85 percent of full stocking. Two replicates were eliminated from the 1960 test because of poor germination and damping-off.

Test seedlings were artificially inoculated under cool, moist conditions induced within large, shaded polyethylene-film inoculation chambers (Figure 3). In all three tests a single chamber covered the entire test. Inoculum was spores of the blister rust fungus, shed from telia borne on the undersides of leaves of naturally infected alternate host plants (wild currants, Ribes spp.). This plant material was collected at several localities in northern Idaho, by merely pruning large, infected-leaf-bearing stems, or by gathering complete currant bushes.

Test seedlings were inoculated in the fall of their first growing season, when they bore only primary foliage, or rarely, secondary (5-needle) foliage. Thereafter, one and two years after inoculation, individual seedlings were inspected to determine whether they exhibited foliar or bark symptoms of the disease. Following the second annual inspection, tallies of the number of healthy seedlings (those
lacking active bark cankers), against the total number of seedlings (those healthy plus those infected), were made for each plot. Proportions — number healthy divided by the total number of seedlings per plot — thus comprised the basic data of the experiments.

Statistical Model and Transformation of Basic Proportion Data

The statistical model assumed that the tester and candidate were random samples from populations described above, and that replications were fixed. The formula for this model was

\[ X_{ijk} = \mu + r_i + c_j + (r_j c_j) + R_k + b_{ijk} + e_{ijk} + d_{ijk} \]  

where

- \( X_{ijk} \) = The transformed proportion of healthy seedlings from the cross of the ith tester and the jth candidate in the kth replication.
- \( \mu \) = General mean.
- \( r_i \) = Effect of the ith tester (\( i = 1,2, \ldots, I \), where \( I = \) total number of testers).
- \( c_j \) = Effect of the jth candidate (\( j = 1,2, \ldots, J \), where \( J = \) total number of candidates).
- \( (r_j c_j) \) = Effect of the interaction of the ith tester and the jth candidate.
- \( R_k \) = Effect of the kth replication (\( k = 1,2, \ldots, K \), where \( K = \) total number of replications).
- \( b_{ijk} \) = Effect of binomial sampling.
- \( e_{ijk} \) = Effect of plot.
- \( d_{ijk} \) = Effect of individuals within plots.

The basic data, that is, the proportion of healthy seedlings per plot, were calculated as follows:

\[ P_{ijk} = \frac{n_{ijk}}{m_{ijk}} \]

where \( n_{ijk} \) was the number of healthy seedlings for the ith tester, crossed to the jth candidate, in the kth replicate; and \( m_{ijk} \) was the total number of seedlings in this group. When \( m_{ijk} \) was small, the percentages fluctuated widely, to make the variance more representative, adjustments were made as suggested by Bartlett (1947, p. 46). If the number of healthy seedlings was equal to the total number of seedlings, then

\[ P_{ijk} = 1 - \frac{1}{4m_{ijk}}. \]

If the number of healthy seedlings was zero, then

\[ P_{ijk} = \frac{1}{4m_{ijk}}. \]

Because the \( P_{ijk} \)'s were binomial, they were transformed to arcsins to stabilize the variance (Bartlett, 1936). Thus,

\[ X_{ijk} = \text{arcsin} \sqrt{P_{ijk}}. \]

Estimates of values for missing plots were necessary. There were 17 missing plots in the 1960 test, 3 in the 1962 test, and 4 in the 1963 test. Values for these missing plots were estimated using the methods of Sverdonos (1957, p. 310), and in the data analyses, appropriate reductions were made in the degrees of freedom.

Estimation of Binomial and Genetic Variances

The initial observations were tallies in which seedlings were assigned a value of 1 if healthy, and 0 if infected. Thus, each seedling had a probability \( p_i \) of being healthy, where \( i \) was the individual seedling in the plot.

For a single plot there were \( m_{ijk} \) test seedlings, and the expected percent healthy seedlings, \( P_{ijk} \), for sample size \( m_{ijk} \) was calculated as follows (for simplicity, here \( m = m_{ijk} \) and \( P = P_{ijk} \)):

\[ P = \frac{1}{m} \sum_{i=1}^{m} P_i \]

with a variance

\[ \sigma^2_p = \frac{1}{m} P (1 - P) \frac{1}{m} \sigma^2_d, \]

where \( P (1 - P) = \sigma^2_b \), and where \( \sigma^2_d \) is the variance due to the inequality of the probabilities \( p_i \), arising from environmental and genetic variation among the seedlings of a plot, that is, the effect of individuals within plots. The variance \( \sigma^2_p \) is composed of binomial sampling variance (1/ \( m \) \( \sigma^2_b \)) minus the within-plot variance (1/ \( m \) \( \sigma^2_d \)) and is at a maximum when all \( p_i \)'s are equal (\( \sigma^2_d = 0 \), Kendall and Stuart 1963, p. 127). When the proportion \( P_{ijk} \) is transformed to arcsin in degrees, the binomial sampling variance becomes a constant equal to 1/ \( m \) 821 (Scheffé 1959, p. 365). Thus from equation 2

\[ \sigma^2_p = \frac{1}{m} \left[ \frac{821}{m} - \frac{1}{m} \sigma^2_d \right]. \]

The analysis of variance for the study, showing expectations of mean squares and formulas for estimating the variance components, is given in Table 1. In calculation of the variance components by these models, the standard errors were obtained from the following formula (Anderson and Bancroft 1952):

\[ S.E. = \sqrt{\frac{2}{c^2} \left( \frac{MS_{ijh}}{f_{ijh}} \right)} \]

where \( c \) = the coefficient of the variance component, \( MS_{ijh} \) = the hth mean square used to estimate the variance component, and \( f_{ijh} \) = the degrees of freedom of the hth mean square.

The mating design used in these experiments was similar to the factorial design of Experiment II of Comstock and Robinson (1952); the genetic model is given in Table 2. Additional components attributable to binomial sampling variance (\( \sigma^2_b \)) and to the variance of the individual's unequal probability of being infected (\( \sigma^2_d \)) were necessary in this design. Also, since in these experiments there was only one replicate per block, \( \sigma^2_e \) and \( \sigma^2_d \) are confounded, and the term \( \sigma^2_p = \frac{1}{m} \sigma^2_d \), estimated by subtracting 1/821 from \( MS_{VCB} \), contains effects of both plots and individuals within plots.

Cockerham (1963, p. 67) lists five assumptions which must be made when this model is used for estimation of genetic
parameters. For the white pine candidates and testers we can assume (1) diploidy and normal Mendelian inheritance (Kihoshoo 1939), (2) lack of maternal effects, and (3) for lack of evidence to the contrary, no linkage of genes associated with resistance, (4) random sampling of candidates and testers, and (5) sampling from noninbred populations.

Regarding the validity of assumption 4, in this study the candidates represent a random sample of the populations of healthy white pine of the region (Figure 1). It should be noted that these trees have already undergone strong natural selection for resistance because they are the survivors of a severe rust epidemic. Tester trees were candidates selected because they exhibited high general combining ability (GCA) for resistance. Thus they had a history of artificial as well as natural selection. Estimates of genetic parameters, therefore, refer only to these or similar candidate and tester populations.

Regarding validity of assumption 5, it was believed that white pine normally arises from outcrossing — because substantial barriers to inbreeding exist in the form of partial self-incompatibility and self-infertility. Also, because of inbreeding depression in natural stands, the inbreds probably seldom attain reproductive maturity (Barnes 1964, Barnes et al. 1962, Bingham and Soullace 1955). Therefore these candidates and tester populations were considered noninbred.

### Table 1. — Model for analysis of variance (testers and candidates assumed random; fixed replications).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Mean squares</th>
<th>Expectation of mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>K−1</td>
<td>MS_R</td>
<td>Σ 1/ m σ_e^2 + 1/ m σ_b^2 + 1/ m σ_d + KEσ_T + KJσ_T</td>
</tr>
<tr>
<td>Testers</td>
<td>I−1</td>
<td>MS_T</td>
<td>σ_e^2 + 1/ m σ_b^2 + 1/ m σ_d + KEσ_T + KJσ_T</td>
</tr>
<tr>
<td>Candidates</td>
<td>J−1</td>
<td>MS_C</td>
<td>σ_e^2 + 1/ m σ_b^2 + 1/ m σ_d + KEσ_T + KJσ_T</td>
</tr>
<tr>
<td>Tester × Candidate</td>
<td>(I−1) (J−1)</td>
<td>MS_TC</td>
<td>σ_e^2 + 1/ m σ_b^2 + 1/ m σ_d + KEσ_T + KJσ_T</td>
</tr>
<tr>
<td>Tester-Candidate combinations</td>
<td>(I(J−1) (K−1)</td>
<td>MS_TCR</td>
<td>σ_e^2 + 1/ m σ_b^2 + 1/ m σ_d</td>
</tr>
</tbody>
</table>

Χ Replications

1) I, J, and K = total numbers of testers, candidates, and replications, respectively.
2) Formulas for estimating individual variance components are as follows:

\[
\sigma_T^2 = \frac{MS_T - MS_{TC}}{KJ} = \text{variance due to testers}
\]

\[
\sigma_C^2 = \frac{MS_C - MS_{TC}}{KI} = \text{variance due to candidates}
\]

\[
\sigma_{TC}^2 = \frac{MS_{TC} - MS_{T} - MS_C}{K} = \text{variance due to interaction of testers and candidates}
\]

\[
\sigma_e^2 = \frac{1}{m} \sigma_d^2 = \text{variance due to effect of plot}
\]

\[
\sigma_d^2 = \text{variance due to effect of individuals}
\]

\[
\sigma_{b}^2 = 821
\]

\[
\text{m} = \frac{1}{\sum_{i=1}^{n} x_i^2} = \text{harmonic mean number of seedlings}
\]

### Table 2. — Genetic model of covariances of relatives.

<table>
<thead>
<tr>
<th>Variance component (^1)</th>
<th>Proportion of variance contributed by each source</th>
<th>Genetic variance</th>
<th>Environmental variance</th>
<th>Binomial variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Additive</td>
<td>Dominance</td>
<td>Epistatic</td>
<td>Between plots</td>
</tr>
<tr>
<td>(\sigma_T^2)</td>
<td>1/4</td>
<td>0</td>
<td>&gt;1/16</td>
<td>0</td>
</tr>
<tr>
<td>(\sigma_C^2)</td>
<td>1/4</td>
<td>0</td>
<td>&gt;1/16</td>
<td>0</td>
</tr>
<tr>
<td>(\sigma_{TC}^2)</td>
<td>0</td>
<td>1/4</td>
<td>&gt;1/8</td>
<td>0</td>
</tr>
<tr>
<td>(\sigma_e^2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(\sigma_d^2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(\sigma_b^2)</td>
<td>1/2</td>
<td>3/4</td>
<td>&lt;3/4</td>
<td>0</td>
</tr>
</tbody>
</table>

1) \(\sigma_T^2\) = cov_{T}, \(\sigma_C^2\) = cov_{C}, \(\sigma_{TC}^2\) = cov_{TC} (covariance of full sibs)

\(\sigma_e^2\) = cov_{e} (covariance of half sibs)

\(\sigma_d^2\) = cov_{d} (covariance candidate half sibs)

\(\sigma_{TC}^2\) = cov_{TC} (covariance candidate half sibs)

\(\sigma_{T}^2 = \text{total genetic variance; } \sigma_{w}^2 = \text{environmental variance within a plot)
the binomial variance, and the two different environmental variances.

The heritability of a tester or candidate tree was calculated according to the following model:

\[ h^2_{(ot)} = \frac{\sigma^2_A}{\sigma^2_T + \sigma^2_P + \sigma^2_{TP} + \sigma^2_g + \frac{1}{m} \sigma^2_h - \frac{1}{m} \sigma^2_d} \]  

where \( \sigma^2_A \) is the additive genetic variance, estimated from 4 \( \sigma^2_T \) or 4 \( \sigma^2_P \). An estimate of the heritability of a specific population on an individual seedling basis was obtained by setting \( m = 1 \). This estimate can be compared with estimates derived from other experiments using different numbers of individuals per plot. However, because success of inoculation — thus intensity of infection — varies between experiments, as do \( \sigma^2_P \) and \( \sigma^2_g \) (environmental variance between plots and within plots, respectively), judgment is required in comparing heritabilities from different experiments (Hanson 1963).

Probably the most useful estimate of heritability would be one based upon the actual materials to be utilized in an operational tree improvement program. In the present experiments, these materials would be the “selection unit,” that is, the four candidate X tester progenies replicated over K blocks with m seedlings per plot. Thus the selection unit comprised 4 families composed of full sibs, each family being half sib in relation to the other tester lines. Heritability when the candidates to be mated, not healthy seedlings from tester matings, were chosen on the basis of performance within this selection unit was

\[ h^2 = \frac{\sigma^2_A}{\sigma^2_T + \sigma^2_P + \frac{\sigma^2_{TP}}{1} + \frac{\sigma^2_g}{m} + \frac{\sigma^2_h}{m} - \frac{\sigma^2_d}{K}} \]  

where \( \sigma^2_A \) is equal to 2 \( \sigma^2_T \), being one-half of the additive genetic variance.

The use of \( \sigma^2_A \) here is based upon the similarity of the mating design of these experiments to that of Roan on et al. (1955), in which, because both parents were selected on the basis of half-sib performance, one-half of the additive genetic variance was used in the numerator of the \( h^2 \) formula. This concept was pursued further by Hanson (1963), and Namkong et al. (1966, in their Case 2) extended it to tester matings.

No variance components expressing genotype-environment interactions were included in formulae (3) and (4). This omission is considered valid because uniformly cultured nursery seedlings were artificially inoculated in a single, large inoculation chamber. With this experimental design and random model, genotype-environment interactions were not estimated but were presumed to be small.

Genetic Gain Analyses

The base population consisted of all western white pines in the area sampled (Figure 1). Successive genetic gains are calculated beginning from this base.

The general formula for estimating genetic gain is

\[ \Delta G = Sh^2 \]  

where \( S \) is selection differential expressed as the mean of the individuals selected to be parents of the next generation, minus the overall mean.

When \( S \) is not known, but the proportion selected is known or postulated, genetic gain is estimated by the formula

\[ \Delta G = Sh^2 \Phi_{phen} \]  

where \( s \) is the standardized selection differential obtained on a mathematical basis (directly from published tables, e.g., Table II, Pearson 1931, or Table II, Becker 1967), and \( \Phi_{phen} \) is the phenotypic standard deviation (square root of denominators of formulas 3 or 4). Because \( \Phi_{phen} \) is in transformed units, the gain would be added to the mean calculated from transformed observations, and the result converted back to percent. The gain in percent is this total minus the percent mean of the population.

When progenies are artificially inoculated and all individual seedlings remaining healthy, or the same proportion of healthy seedlings from each mating, are planted to establish seed orchards, formula (6) gives the gain expected in the seed orchard progeny. The \( s \) is determined from the proportion of healthy seedlings, and this proportion is obtained by adding all genetic gains up to the particular point reached in the breeding program. The heritability and \( \Phi_{phen} \) are those of formula (3).

Estimation of Combining Ability

The GCA of each parental tree, the specific combining ability (SCA) of each tester-candidate cross, and the SCA variance were estimated, the parents being assumed to be chosen or fixed. The model given by Griffing (1956, Method 4) was adapted to a factorial design.

The statistical model was

\[ \bar{x}_{ij} = \bar{x} + g_i + e_i + \frac{b_{ij} + e_{ij}}{K} \]  

where \( \bar{x}_{ij} \) is the mean of the \( i \)th tester crossed to the \( j \)th candidate over \( K \) replicates and transformed as in the previous model; \( \bar{x} \) is the general mean; \( g_i \) is the GCA associated with the \( i \)th tester; \( e_i \) is the GCA associated with the \( j \)th candidate; \( e_{ij} \) is the SCA associated with the cross of the \( i \)th tester and \( j \)th candidate; \( b_{ij} \) is the binomial sampling effect; and \( e_{ij} \) is the residual effect particular to the \( i \)th and \( j \)th cross.

The computational formulae were as follows:

**Tester Trees**

\[ \text{GCA}_i = \bar{x}_{i} - \bar{x} \]  

\[ \text{SCA}_{ij} = \bar{x}_{ij} - \bar{x}_i - \bar{x}_j + \bar{x} \]  

\[ \sum_{j}^{2} \text{SCA}_{ij} = \frac{\sum_{j}^{2} \text{MS}_{TCR}}{K} \]  

**Candidate Trees**

\[ \text{GCA}_j = \bar{x}_j - \bar{x} \]  

\[ \text{SCA}_{ij} = \bar{x}_{ij} - \bar{x}_i - \bar{x}_j + \bar{x} \]  

\[ \sum_{i}^{2} \text{SCA}_{ij} = \frac{\sum_{i}^{2} \text{MS}_{TCR}}{K} \]  

Results

Percent of Healthy Seedlings

Mean percentages of healthy seedlings for individual testers, and for candidates or controls as a whole, calculated from plot values that were neither adjusted nor transformed, are shown in Table 3. There were relatively wide differences between the three tests in percents of seedlings remaining healthy in both the candidate and tester results. However, results with greater similarity (7.1, 5.6, and 4.3 percent) were obtained when percents of healthy control seedlings were subtracted from percents observed for candidates. The assumption was that healthy control seed-
Table 1. — Mean total number of seedlings and percent healthy seedlings per plot for three progeny tests.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>1960</th>
<th>1962</th>
<th>1963</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean total number (m)</td>
<td>13.6</td>
<td>11.8</td>
<td>12.0</td>
</tr>
<tr>
<td>Mean percent healthy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testers 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>36.1</td>
<td>9.8</td>
<td>10.8</td>
</tr>
<tr>
<td>b</td>
<td>37.5</td>
<td>9.5</td>
<td>8.7</td>
</tr>
<tr>
<td>c</td>
<td>33.5</td>
<td>5.9</td>
<td>13.4</td>
</tr>
<tr>
<td>d</td>
<td>35.5</td>
<td>8.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Candidates (X ...)</td>
<td>35.7</td>
<td>8.5</td>
<td>11.4</td>
</tr>
<tr>
<td>Controls (base population)</td>
<td>28.6</td>
<td>2.9</td>
<td>7.1</td>
</tr>
<tr>
<td>Gain over base population</td>
<td>7.1</td>
<td>5.6</td>
<td>4.3</td>
</tr>
</tbody>
</table>

1) In the 1960 and 1962 experiments testers were identical; in 1963 a different set of testers was used.

Table 4. — Analysis of variance of adjusted and transformed data.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>1960</th>
<th>1962</th>
<th>1963</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>M.S.</td>
<td>d.f.</td>
</tr>
<tr>
<td>Replications</td>
<td>7</td>
<td>13,381.16</td>
<td>9</td>
</tr>
<tr>
<td>Testers</td>
<td>3</td>
<td>738.93*</td>
<td>3</td>
</tr>
<tr>
<td>Candidates</td>
<td>51</td>
<td>874.77**</td>
<td>35</td>
</tr>
<tr>
<td>Tester X Candidate</td>
<td>153</td>
<td>222.57</td>
<td>105</td>
</tr>
<tr>
<td>Tester-Candidate combinations</td>
<td>1,432</td>
<td>231.33</td>
<td>1,284</td>
</tr>
</tbody>
</table>

1) Degrees of freedom were reduced for missing plots as follows:  
* Significant at the 5% level of probability.
** Significant at the 1% level of probability.

Frequency histograms (Figure 4) show the range in percent of healthy seedlings found in progenies of the various candidates of the three tests. These histograms show that response to the disease falls into a normal distribution, although the 1960 test data show some bimodality.

Heritability Analyses

For the remaining analyses plot values were adjusted and transformed as described in the materials and methods section. The analyses of variance based on these adjusted and transformed data are shown in Table 4. Mean squares for testers and candidates were significant in all three experiments. Mean square for tester X candidate interaction was significant in the 1962 and 1963 experiments. Components of variance obtained from the mean squares in Table 4 are presented in Table 5, along with their standard errors, and heritability estimates calculated from these components, along with their standard errors, are given in Table 6.

Heritability estimates are presented both on an individual seedling basis (that is, with m arising from binomial sampling variance equaling 1 seedling, formula 3) and on a selection unit basis (formula 4). The estimate \( \sigma_T^2 \) contains at least one-quarter the dominance and one-eighth the epistatic variance. Therefore, on a biological basis it cannot be negative, and in the 1960 experiment the negative value (-1.12, Table 5) was assumed to be zero in the heritability calculations. The negative value for \( \frac{1}{m} \sigma_e^2 - \frac{1}{m} \sigma_d^2 \) in the 1962 experiment (-5.13, Table 5) would occur when the variation within a plot was greater than that among plots.

Heritability for the selection unit being used in the present program exceeded heritabilities for an individual seedling.

Combining Ability Analyses

Values for combining ability identify candidates having superior progeny (high GCA), which is the criterion for selection. Also, values for specific combining ability (SCA) provide information on the performance of candidates in combination with specific tester trees. Results of analysis
for GCA and SCA for the 15 percent of the candidates having the highest GCA are presented in Table 7. The values were obtained from the data on percent of healthy seedlings, with the exception of $\sigma_{S_{SCA}}^2$, which was calculated from adjusted and transformed plot values. Negative $\sigma_{S_{SCA}}^2$ estimates were obtained for the 1960 experiment because the $MS_{PCR}$ (Table 4) and the $\sigma_{e}^2 = \frac{1}{m} \sigma_{d}^2$ values (Table 5) were high in that experiment.

**Genetic Gain**

There are three increments of expected genetic advance in this breeding program. The first comes from the selection of rust-free candidates from within the generally susceptible base population. Here this increment is expressed as the percent of healthy seedlings in the average candidate X tester mating minus the percent healthy in the base population (controls), although this is an overestimate of gain because testers have been selected for high GCA. Estimates were 7.1 percent in 1960, 5.6 percent in 1962, and 4.3 percent in 1963 (Table 3).

The second increment comes from selective mating of high-GCA candidates as opposed to all candidates (formula 5). In Table 8 these gains are listed under $\Delta G_{I}$. They are underestimated because the overestimate of first-increment gains reduces $S$ in formula (5).

The third increment comes from selection of all or proportionate numbers of healthy seedlings within high-GCA candidate progenies, after their artificial inoculation, eventually followed by mating of the selected seedlings in seed orchards (formula 6). These gains are shown in Table 8 under $\Delta G_{I}^{}$.

The total advance above the base population is the sum of these three increments, shown in Table 8 as the total gain above base.

**Discussion**

**Levels of Resistance**

Perhaps the most striking results of the three experiments were the low levels of resistance observed for average candidate X tester progenies (Table 3). After the percent of healthy seedlings in the base population was deducted from that in the candidate population, an advantage of only 4 to 7 percent was evident. However, two features of these tests tended to reduce differences observed between candidate and control (base population) progenies.

First, progenies were inoculated only when one year old, in a stage now known to be highly susceptible. It is quite clear that resistance to blister rust increases with age of seedlings at time of exposure to the rust (HEIMBURGER 1938, 1960, 1962).

### Table 7. Means and combining ability of top 15 percent of the candidate trees, expressed in percent healthy seedlings for three experiments.\(^1\)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Candidate No.</th>
<th>Overall mean (^2)</th>
<th>Performance</th>
<th>Tester cross performance(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GCA</td>
<td>$\sigma_{S_{SCA}}^2$</td>
<td>Tester a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SCA</td>
<td>Mean</td>
</tr>
<tr>
<td>1960</td>
<td>151</td>
<td>55.6</td>
<td>19.9</td>
<td>-12.8</td>
</tr>
<tr>
<td></td>
<td>93</td>
<td>55.5</td>
<td>19.9</td>
<td>-16.8</td>
</tr>
<tr>
<td></td>
<td>118</td>
<td>53.3</td>
<td>17.6</td>
<td>-12.9</td>
</tr>
<tr>
<td></td>
<td>117</td>
<td>50.8</td>
<td>15.2</td>
<td>-6.8</td>
</tr>
<tr>
<td></td>
<td>147</td>
<td>47.6</td>
<td>11.9</td>
<td>-9.3</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>45.9</td>
<td>10.2</td>
<td>-6.7</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td>45.2</td>
<td>9.6</td>
<td>-6.7</td>
</tr>
<tr>
<td>1962</td>
<td>212</td>
<td>44.2</td>
<td>8.5</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>222</td>
<td>19.1</td>
<td>10.6</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>17.0</td>
<td>8.5</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>264</td>
<td>16.0</td>
<td>7.5</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>272</td>
<td>13.1</td>
<td>4.6</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>12.5</td>
<td>4.0</td>
<td>37.4</td>
</tr>
<tr>
<td>1963</td>
<td>364</td>
<td>20.4</td>
<td>9.0</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>322</td>
<td>19.3</td>
<td>7.9</td>
<td>-8.0</td>
</tr>
<tr>
<td></td>
<td>342</td>
<td>17.9</td>
<td>6.6</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>358</td>
<td>17.4</td>
<td>6.1</td>
<td>28.1</td>
</tr>
<tr>
<td></td>
<td>341</td>
<td>16.2</td>
<td>4.8</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>303</td>
<td>13.9</td>
<td>4.5</td>
<td>11.8</td>
</tr>
</tbody>
</table>

\(^1\) See Table 3.\(^2\) See Table 3 for percent of healthy seedlings in the controls (base population).\(^3\) Unweighted average percents from the 45 plots (32 plots for 1960, after elimination of 2 replicates).\(^4\) In the 1960 and 1962 experiments testers were identical; in 1963 a different set was used.\(^5\) $\sigma_{S_{SCA}}^2$ calculated with adjusted and transformed plot values: $\sigma_{e}^2$ in percent.
Table 8. — Expected genetic advance from crossing superior candidates and from selection of superior individuals, at various degrees of selection intensity.

<table>
<thead>
<tr>
<th>Candidates selected</th>
<th>1960</th>
<th>1962</th>
<th>1963</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approx. number of candidates</td>
<td>5Gc(^1)</td>
<td>5Gc(^3)</td>
<td>5Gc(^3)</td>
</tr>
<tr>
<td>2</td>
<td>10.4</td>
<td>5.4</td>
<td>11.1</td>
</tr>
<tr>
<td>6</td>
<td>7.1</td>
<td>4.2</td>
<td>9.9</td>
</tr>
<tr>
<td>9</td>
<td>5.9</td>
<td>3.6</td>
<td>9.3</td>
</tr>
<tr>
<td>11</td>
<td>5.2</td>
<td>3.2</td>
<td>8.9</td>
</tr>
<tr>
<td>21</td>
<td>3.9</td>
<td>1.9</td>
<td>7.6</td>
</tr>
</tbody>
</table>

\(^1\) Genetic gain from selective mating of high-GCA candidates.

\(^2\) Genetic gain from selection and mating of seedlings from high-GCA candidate progenies.

\(^3\) Includes gain from selection of rust-free candidates from the base population: 7.1 percent for 1960, 5.6 percent for 1962, and 4.3 percent for 1963.

Paton 1961, Patton and Riker 1966, unpublished data from this project. In fact, where inoculations are made too early in seedling life, resistance may be almost completely masked by overriding influences of heavy infection. Patton and Riker (1968) showed that resistance types inoculated at age one were found to contain less than one percent of disease-free seedlings. But after inoculation at age four, identical progenies were found to contain about 20 to 30 percent disease-free seedlings, while controls of the same age were reduced to 1 percent disease-free.

Second, the interval from inoculation to final rust inspection as reported here was probably too short to provide the most meaningful results. It was desirable to include three experiments in these analyses, and to maintain uniform experimental conditions throughout. Therefore, the interval between inoculation and inspection was set at only two years, because the most recent (1963) test had not progressed beyond that stage. This short interval does not provide a complete picture of rust development because (a) latent infection of control seedlings is greater than that of candidate seedlings, and (b) postinfectional resistance mechanisms, particularly those seated in the bark (Bingham et al. 1960), have not had time to come into play.

For instance, in the 1960 and 1962 tests of this paper, and in one earlier test, wherein percentages of healthy seedlings could be observed at three as well as at two years after inoculation, third-year percentages dropped far more for control progenies than for progenies of the better candidates. This second- to third-year decrease in healthy seedlings for controls against candidates is shown below.

<table>
<thead>
<tr>
<th>Year of test</th>
<th>Control progenies</th>
<th>Progenies of best 15 percent of candidates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent decrease</td>
</tr>
<tr>
<td>1952</td>
<td>5</td>
<td>13.7</td>
</tr>
<tr>
<td>1960</td>
<td>10</td>
<td>24.8</td>
</tr>
<tr>
<td>1962</td>
<td>6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

The net effect of greater latent infection of controls and latent resistance of candidate seedlings was to increase observed levels of resistance of candidate progenies over the base population. Whereas, at two years after inoculation in the 1962 test, progenies of the better candidates contained, on the average, 7.2 percent more healthy seedlings than controls, at three years the difference had increased to 13.3 percent (and by 7 years it was 28.4 percent). Corresponding data for the 1960 test were 21.2 percent at two years and 29.0 percent at three years; for the 1962 test they were 12.6 percent at 2 years and 12.9 percent at 3 years — even though controls were already reduced to 2.9 percent healthy at 2 years (Table 3).

Thus the authors wish to point out that proportions of healthy seedlings observed in these experiments are probably below those that will hold for older nursery seedlings, and especially for 3-year or older nursery stocks coming from seed orchard nurseries. Within a few years we will be able to check the validity of these assumptions. Experiments to appraise effect of age of seedlings at time of inoculation are underway, and present experiments will be examined up to 3 years after inoculation to appraise effects of latent infection and resistance. Meanwhile, data covered here will serve to establish methodology for estimating heritability and genetic gains.

Another interesting feature of the results given in Table 3 is the variation in observed levels of infection between experiments. Differences of 20 to 25 percent in resistance were observed between the base (control) population of the 1960 experiment and the two later experiments. Apparently they arose because of variation from one test to another in effectiveness of inoculation, but if so, changes in the potency of inoculum or of other likely factors remain unexplained. In fact, in the 1960 test, where infection was lightest, two to three times more basidiospores were trapped on spore-cast slides than in the two later tests, in which infection was higher.

There was also definite genetic variation within each experiment.

Figure 4 shows that mean percents of healthy seedlings observed for the progenies of each candidate varied rather widely; actual values are from 17 to 56 in the 1960 experiment, 1 to 19 in 1962, and 3 to 20 in 1963. Both candidates and testers have a significant influence on progeny resistance (Table 4).

Genetic Variances

Estimates of variance components for testers were much lower than those for candidates (Table 5), probably because testers had already been selected for high GCA.

Additive genetic variance for the candidate population (4 × c\(^2\)) changed from year to year, being 81.52 ± 2.48 in 1960, 41.44 ± 12.24 in 1962, and 21.36 ± 7.82 in 1963 (Table 5 or 6). The main reason for these changes is believed to be the changing levels of infection in the three experiments, although the different candidates involved in the experiments may have caused some shift.
The estimate of dominance variance and half the epistatic variance (\(s^2_{DI} \times 4\), Table 6) was essentially zero in 1960, 10.84 in 1962, and 11.20 in 1963.

Under the assumption that resistance is controlled by polygenes and that environmental influences vary at random across plots, there would be an underlying, continuous variation in resistance, both genetic and environmental. Imposed on this hidden, continuous scale of variation is a visible, but discontinuous scale of variation, that is, proportions of seedlings healthy and diseased. In each experiment the two scales have one point in common — a threshold above which average phenotypes remain healthy, below which they become infected (see Falconer 1960, pp. 301—302). Thresholds in the three experiments are exemplified by mean percentages of healthy seedlings (of candidates, testers, or controls) in Table 3.

Where inoculation is highly successful and results in intensive infection, the threshold value moves toward zero. Only those seedlings having the greatest resistance remain healthy. Under these conditions the combined estimate for dominance and epistatic variance is expected to increase relative to additive genetic variance (Dempster and Lerner 1950).

Infection levels were higher in the 1962 and 1963 experiments than in 1960, and dominance plus epistatic variances were also greater in those two years than in 1960, probably because of this effect. Thus heterosis might not be a factor under the lighter levels of infection anticipated for older seedlings or plantation trees; but it could become quite important when susceptible young seedlings were heavily infected.

In addition, as the level of infection becomes more uniform across all plots — possibly through improved inoculation technique — values for the terms \(s^2_e\), (plot effect) and \(s^2_i\), (effect of individuals within plots) may be expected to decrease. Decrease in \(s^2_e\), will reduce the denominator in the heritability equations (formulas 3 and 4), whereas a decrease in \(s^2_i\), will increase the denominator. The net effect of changes in these two terms may either increase or decrease the heritability in experiments with more uniform inoculation.

Heritability Estimates

The heritability estimates were low for the individual seedlings but were much higher for the selection unit (Table 6). These low individual seedlings estimates reflect the slow gain to be made under natural selection in the forest, where selection for resistance is on an individual tree basis.

Genetic Gains under Various Breeding and Seed Orchard Schemes

The breeder will need to take into account planting adaptation and inbreeding in planning and establishing seed orchards.

Seed orchard entries should be controlled so that anyone using planting stocks from the orchards can expect the stock to grow reasonably well on the planting sites involved. Thus in this western white pine program three elevational-zone orchards will be utilized in an attempt to control significant effects of clinal variation in growth associated with elevation.

A genetic base sufficiently broad to avoid inbreeding depression should be maintained. Srenk (1959), for example, recommends that no fewer than 28 to 30 parents be entered in clonal orchards, or 30 to 40 if selection is to be continued. Thus each of the zone orchards will contain progeny of approximately 25 high-GCA candidates.

The breeder cannot utilize the SCA variance estimates for the candidates (Table 7) in the early stages of the program. Practical use of SCA must await further information on its possible significance.

Degree of genetic gain attained will vary according to selection and testing criteria imposed by the breeder. A summary of the alternatives is given in Figure 5. A certain amount of gain will be realized when rust-free candidates are selected in the forest and seed orchards are established with grafts of the candidates. Selection Stage A, and Stage A orchards of Figure 5 illustrate this concept and the expected gain. The gain is difficult to estimate in these experiments because all matings involved testers chosen for high GCA for resistance. Depending on the conditions of the experiment, however, gain would be somewhat below 4.3 to 7.1 percent over base population of Table 3. Here only 72 to 90 candidate trees would have to be found to provide grafts to establish three 24- to 30-clone orchards, and 2 or more years would be required to find and graft the candidates.

As shown in Figure 5, Stage B, selection of the best 15 percent among the high-GCA candidates, based on progeny performance, followed by establishment of grafted orchards from these candidates, would produce a net gain of 8.5 to 19.9 percent above the base population (gain from Table 3 plus \(G_{GC}\) gain, Table 8). At Stage B, seed orchards could be established without further selection. Here at least 480 to 600 candidates would be required in the genetic base, and 7 or more additional years would be required to find and test the candidates.

Selection Stage C would require the longest time (one additional 6-year test cycle) but would provide the largest genetic gains. The high-GCA candidates would be mated — up to 15 pairs, both parents being chosen from the same elevational zone, for 3 zones — and their progenies would be exposed to artificial inoculation. Remaining healthy seedlings, or a proportional number of healthy seedlings, would be planted to establish Stage C orchards. The total genetic gain is estimated as 9.9 to 23.7 percent (Stage B gain plus \(G_{GC}\), Stage C).

STAGE A: SELECTION OF CANDIDATE TREES

Stem three best untested trees from heavily selected natural stands

STAGE B: SELECTION OF HIGH-GCA CANDIDATES

Based on levels of resistance found in artificially inoculated tester X candidate progeny

STAGE C: SELECTION OF REBRENT SEEDLENS OF HIGH-GCA X HIGH-GCA MATINGS

Best 15 percent of the GCA candidates based within three elevational zones, progeny artificially inoculated and healthy seedlings retained

STAGE A ORCHARDS

Grants from all candidates, 24 to 30 for each of three elevational-zone orchards. Genetic base: 72-90 candidate trees required. Time: 3 years or more. Expected gain: < 4.2-7.1 percent above base populations (Table 3)

STAGE B ORCHARDS

Grants from the best 15 percent of high-GCA candidates, 24 to 30 for each of three elevational-zone orchards. Genetic base: 480-600 candidate trees required. Time: 7 years or more. Expected gain: 8.5-19.9 percent above base population (Tables 3 and 8)

STAGE C ORCHARDS

The same propagation of healthy seedlings from high-GCA X high-GCA matings. 15 to 15 progenies (G3) candidates for each of three elevational-zone orchards. Genetic base: 480-600 candidate trees required. Time: 15 years or more. Expected gain: 9.9-23.7 percent above base population (Table 8)

Figure 5. — Alternative breeding and seed orchard establishment schemes, with required base in candidate trees and expected genetic gains.
Table 8 shows that the smaller the number or proportion of the high-GCA candidates selected, the larger the genetic advance ($\Delta G_c$). It is also apparent that there is a direct correspondence between a large $\Delta G_c$ and a small gain from selecting individuals ($\Delta G_{ind}$). The reason is that at high $\Delta G_c$ a larger proportion of the seedlings would remain healthy for selection and planting in Stage C orchards, resulting in a lower selection differential for individuals.

Physical and financial limitations imposed by the number of candidates it is possible to find and test, as well as the need to maintain a genetic base broad enough to prevent inbreeding depression, might require that a higher proportion of the GCA candidates be selected and a lower total gain accepted in Stage B or C orchards.

Although these genetic gains are modest, and although it is impossible to predict with certainty that they would be greater or smaller under periodic, though lighter, natural inoculation in field plantings, it seems reasonable to expect greater gains there. Reasons for expectation of better field performance are that (1) the 3- to 4-year-old stocks coming from seed orchard nurseries should have a generally higher level of resistance (above controls) than the 1-year-old seedlings tested here, and that (2) in other experiments after 12 years of exposure to rust on field plots where Ribes spp. were planted not over 6 feet from all test trees, small first-generation progenies from matings of high-GCA parents survive at levels 15 to 25 percent above those estimated for similar (Stage B) progenies from these experiments.

One way to increase gains would be to select still smaller proportions of the high-GCA candidates. This could be accomplished with roughly the same outlays, if larger numbers of candidates were tested with smaller numbers of plots per candidate. Under the assumption that 10 candidates will be selected regardless of the total number tested, and given a fixed test nursery size in each experiment, then using formula (6), it is possible to estimate the optimal number of candidates to test to secure the greatest gain. Increasing the number of candidates tested in a nursery of fixed size reduces the number of plots per candidate, and thus reduces selection unit heritability. On the other hand, selecting a smaller proportion of the better candidates increases selection differential.

Net effect of these factors for each of the three experiments is shown in Figure 6. The curves suggest that about 60 candidates, and probably no fewer than 40, should be entered in each test; also, approximately 7 plots per candidate $\times$ tester mating would be sufficient. Continuation of the curves indicated that gain decreased above 80 to 120 candidates per test, or at 5 to 3% plots per candidate.

Savings in the cost of progeny testing for estimation of GCA can also be made by effecting other improvements in test design, inoculation, and pollenization procedures (Cf. Bingham 1968).

Summary

Genetic variances, heritabilities, and genetic gains under selection for blister rust resistance in western white pine were obtained in three experiments. Healthy trees surrounded by diseased trees in natural forest stands of northeastern Idaho and adjacent states constituted the main reference population.

These "candidates" were mated to four tester trees, and the progenies were planted in a randomized block design. One-year-old seedlings progenies were artificially inoculated with the rust fungus; the number of seedlings remaining healthy in each of 8 to 10 small plots per candidate $\times$ tester mating was determined in each of the three experiments two years after inoculation. Percent of healthy seedlings per plot averaged 35.7, 8.5, and 11.4 in the three experiments.

Individual candidate-seedling heritabilities were 8.5, 5.0, and 2.0 percent, and heritabilities of selection units (full- and half-sib families from mating candidates with four testers) were 82.2, 106.8, and 64.4 percent. Dominance and epistatic variances were high in the two most heavily infected experiments. General combining ability (GCA) in the best 15 percent of the candidates ranged from about 4 to 29 percent healthy seedlings.

Genetic gain from selection of all candidates was 7.1, 5.6, and 4.3 percent healthy seedlings, and selection of the best 15 percent among candidates exhibiting GCA (on basis of progeny performance) gave additional gains of 12.8, 7.1, and 4.2 percent. Selection on an individual seedling basis, whereby progenies of the best 15 percent of the GCA candidates were artificially inoculated and the remaining healthy individuals were used for establishment of seed orchards, gave still further gains of 3.8, 2.9, and 1.4 percent.

Utilizing all three methods of selection, total gains were estimated at 23.7, 15.8, and 9.9 percent above the base population.

For best results, at least 40 and not more than 80 candidates should be tested per experiment. Actual percent of seedlings remaining healthy under natural inoculation of seed orchard progenies in the field probably will be greater than indicated by these experiments.

**Literature Cited**

Contributions of Tops and Roots to Variation in Height Growth of Geographic Sources of Shortleaf Pine

By Robert M. Allen

(Received for publication November 15, 1967)

Introduction

Studies such as Wells and Wakeley's (1965) with Pinus taeda L. have demonstrated a relation between climate at the seed source and height variation among provenances. Because the selection pressures on the above-ground tree parts are not necessarily the same as those on the roots, the tops and roots may not contribute equally to such variation. The present study was designed to determine the possible contributions of the tops and of the roots to the variation in height growth attributable to geographic origin of seed. It represents a continuation of efforts to assess the variation in height growth of southern pines and the contribution of tree parts to this variation (Allen, 1964 and 1967).

Methods

Tops of seedlings from seven geographic seed sources of shortleaf pine (P. echinata Mill.) were grafted on seedling rootstocks from their own source and a local source (Stone County, Mississippi), and on slash pine (P. elliottii Engelm.) rootstocks. Local scions were also grafted onto rootstocks from the other six sources. Heights of tops were compared after 4 years in the field in this study conducted near Gulfport, Mississippi.