Breeding Blister Rust Resistant Western White Pine

II. First Results of Progeny Tests Including Preliminary Estimates of Heritability and Rate of Improvement[1]

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Introduction

Breeding for resistance to the blister rust fungus (*Cronartium ribicola* FISCHER) in western white pine (*Pinus monticola* DOUGL.) began in Northern Idaho in 1949. A previous progress report in this series, (BINGHAM, SQUILLACE, and DUFFIELD, 1953) outlined selection and progeny test procedures we would use toward obtaining rust-resistant planting stock. This report gives first results on transmission of rust resistance, as shown by performance of control- and wind-pollinated progenies of 21 phenotypically resistant parent selections. It also includes some tentative estimations on heritability of rust resistance and expected rate of improvement per generation of breeding.

We have given particular emphasis to methods of making heritability and improvement estimates using heterogeneous, early-generation materials, for in forest tree breeding an early understanding of the efficacy of selection, as well as foreknowledge of the potential degree of improvement per generation of breeding, is of great practical importance. Gains from forest tree improvement will materialize much more slowly than those made in crop breeding. They are geared to the much slower succession in tree generations and often to a longer period necessary for expression of mature-tree characteristics. It follows that heavy investments in time, funds, and lands will accumulate during the period required for producing and testing a single generation of trees. Early assurance that selection is both realistic and profitable, and that satisfactory improvement will probably accompany future breeding work, is a necessity.

Materials and Methods

Selection of Parents

At the start of selection work we decided to concentrate our efforts on a single characteristic — resistance to blister rust disease. We did this for 4 reasons. First, without blister rust control, management of western white pine is impossible. Second, resistance to the disease is quite easy to recognize in the field in comparison with other characteristics like rate of growth. Third, many of the western white pine stands in which we worked are heavily infected, and the presence of rust-free individuals therein is a good indication that genetic variability in resistance to the disease exists. Fourth, even with the most efficient selection methods (like the "tandem" method), the rate of genetic improvement in any one characteristic is only $1/\sqrt{n}$ times as great when n characteristics are selected for simultaneously as when one characteristic is considered.

The selection process itself was simple (BINGHAM, et al., 1953). It consisted of touring many heavily infected white pine stands in northern Idaho and northwestern Montana and searching out those rare trees having no blister rust cankers (or in a few cases, where neighboring trees had thousands of cankers, those with only 1 or 2 infections). We had help in this selection work. Other members of the Forest Service and lumber industry personnel were on the lookout for rust-free trees and gave us the location of several promising "candidates" and heavily infected selection areas. Inspection from the ground was insufficient, because it was too easy to overlook small cankers high in the crown. Each of the candidates has been climbed and inspected several times before being classified as rust-free.

To date, we have located more than 200 of these rust-free phenotypes. Data from progenies of 21 of these selected trees are included in this paper. Eighteen of the candidates, represented by 2 or more control-pollinated progenies, are considered in more detail in heritability and improvement analyses.

Controlled Pollination

Before entering upon genetic testing of the rust-free phenotypes, we had to make two decisions — whether to concentrate on clonal testing (cf. SYRACH LARSEN, 1947; TODA, 1958) or on the testing of sexual progenies, and whether to concentrate on testing of wind-pollinated progenies or on control-pollinated progenies from 2-parent matings. Sexual progeny testing was believed necessary because clonal propagation is difficult in western white pine (BINGHAM, et al., 1953); 2-parent matings were indicated because their analysis for partitioning genetic variance into additive and nonadditive components is likely to be much more sensitive. Clones can only demonstrate the presence of genetic variation, not its inheritance, and in a seed propagated species we are interested in the performance of seedling progenies. Controlled pollination experiments require a greater initial outlay in time and materials, but do not require progenies as large as those for wind pollination experiments. Furthermore, at the time these experiments were started (and even today), there were no data showing the relative amounts of additive variance, dominance variance, and epistatic variance involved in the inheritance of resistance or other characters of forest trees. Thus, the conlrolled pollination work was most advantageous for determining the pattern of future work programs. In this respect, a diallel crossing program appeared desirable, but even with 21 parents (210 2-parent crosses in the diallel scheme) and the necessity of pollinating 5 or more shoots per cross, it was simply beyond the female flowering capacity of the parent trees. Instead, pollination proceeded to the limit of female flowering, and was thus concentrated largely on several heavily flowering trees.

The progenies reported in this paper resulted from pollinations made in the spring of 1950 according to methods outlined previously (BINGHAM, et al., 1953). It is unnecessary here to describe the details of the technique except to mention that unpollinated controls left on certain female

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Figure 1. — *A: Pinus monticola* 5-needle bundle with 4 typical blister rust needle lesions *(on the left).* — *B:* Single rust-infected needle of *P. monticola* and typical, swollen incipient canker at base of needle *(on the right).*

parents failed to set sound seed; this indicated that bagging precautions had been adequate. Seeds were harvested in the fall of 1951.

Nursery, Outplanting, and Testing Procedures

Seeds were sown in an experimental nursery at Spokane, Washington, in the spring of 1952. In the fall of 1953, the seedlings were artificially inoculated with sporidia of the rust fungus. In the spring of 1954, seedlings were outplanted at 1 × 1-foot spacing in three field plantings near Clarkia, Idaho; Elk River, Idaho; and Saltese, Montana, in the heart of the white pine country.

This experiment is noteworthy in that each seedling occupied the same position relative to other seedlings in the outplanting as in the nursery. That is, progenies of row plots 1, 2, 3, etc., in the nursery also occupied row plots 1, 2, 3, etc., respectively in the outplanting; the same was true in respect to individual trees within the 10-seedling row plots (the basic replicates). The only difference in the nursery and outplanting plots was that the trees which were 2 inches apart in the nursery in 1952 and 1953 were 1 foot apart in the outplantings in 1954 and later years.

The seedlings were artificially inoculated with sporidia of the rust fungus in the fall of 1953 and have been exposed to heavy natural inoculation from interplanted ribes on the outplanting plots since the fall of 1954. Infection was extremely heavy. During the first 2 years after artificial inoculation, the presence of the fungus was detectable externally only by the presence of small, yellow foliage lesions or "needle spots". Through the fall of 1957, when final scorings were made, the presence of the fungus could also be determined from bark cankers. In 1957, seedlings were classified in 5 types: those with no symptoms, those with needle spots only *(figure 1)*, those with inactive bark cankers — resulting either from rapid collapse of the invaded host tissues in what appears to be a hypersensitivity reaction *(figure 2)* or from "corking out" of the invading fungus by the production of wound cambia *(Figure 3*, cf. also Struckmeyer and Riker, 1951), those with active bark cankers, and those that died as a result of bark cankers. For purposes of these analyses, however, only 2 easily recognized classes of seedlings were scored. They were: (1) living seedlings, presumably adequately exposed to the rust fungus, without cankers or with inactive cankers, and (2) living seedlings with active cankers or seedlings that died because of bark cankers. All the data that follow are based on the separation of the 6-year-old seedlings into one or the other of these 2 easily recognizable classes, hereafter called survivors and nonsurvivors.

Experimental Design and Statistical Analysis

In both the nursery and outplantings a randomized block design with 9 replications was used. In each replication every 2-parent mating was represented by a row plot of 10 trees. Mortality due to failure of germination of seed, damping-off of young seedlings, transplanting, rodents, and causes other than blister rust reduced the number of seedlings to an average of 8.96 per row, or 80.6 per mating (progeny).

In the final 1957 examination, the numbers of trees with no, or with only inactive, bark cankers and the total number of trees considered as adequately inoculated were recorded in each 10-seedling plot. Prior to making statistical analyses of the data, the frequencies of percentages of survivors, and of arcsines of percentages of survivors were plotted. The percentage frequency curve was skewed to the left, whereas the arcsine frequency curve was more nearly normal. Accordingly, all percentages were converted to arcsines and all statistical analyses were based on arcsines of percentages.

Analyses of variance were performed on the arcsines of the plot mean percentages of survivors according to the standard methods outlined by Snedecor (1946). These analyses were carried one step further than is usual in forestry analyses by the calculation of expectation mean squares (Snedecor, 1946; Kempthorne, 1957). Although this is a standard statistical procedure in crop genetics, it has been relatively little used with forest tree materials (except by Toda, 1958 and 1959, with clonal or wind-pollinated materials), and for that reason is outlined in detail in the keyout below. In the *keyout*, F, M, and R, are the sources of variation associated, respectively, with female parent, male parent, and replication. The numbers of different females, males, and replications representing each source of variation are denoted by the letters f, m, and r. For excample, if F represents female parent and f equals 10, then 10 female parents are represented in the test. By following the keyout, the components of variance can be obtained by substitution, subtraction, and division.

Keyout: Expectations of mean squares for a three-way classification

Source of variation	Degrees of freedom	Mean square is an estimate of:
F	$(f-1)$	$\sigma^2 + r\sigma^2_{fm} + m\sigma^2_{fr} + mr\sigma^2_f$
M	$(m-1)$	$\sigma^2 + r\sigma^2_{fm} + f\sigma^2_{mr} + fr\sigma^2_m$
R	$(r-1)$	$\sigma^2 + m\sigma^2_{fr} + f\sigma^2_{mr} + fm\sigma^2_r$
FM	$(f-1)(m-1)$	$\sigma^2 + r\sigma^2_{fm}$
FR[1]	$(f-1)(r-1)$	$\sigma^2 + m\sigma^2_{fr}$
MR[1]	$(m-1)(r-1)$	$\sigma^2 + f\sigma^2_{mr}$
FMR	$(f-1)(m-1)(r-1)$	σ^2

[1] Unless experience indicates these error terms differ, they may be omitted, as is done in subsequent analyses.

In partitioning the variance from the analyses of certain partial diallel crossing schemes, we encountered no difficulty in computing the number of female or male parents, because every female parent was crossed with every male parent. However, there was a problem in computing the numbers of female and male parents in the analysis of the data from the complete crossing program. For that analysis, the effective number of female parents (f) was computed as the harmonic mean of the number of females crossed with each male; similarly, the harmonic mean of the number of males mated with each female was used as the mean number of males (m) (cf. SNEDECOR, 1946).

Heritability (H) has been defined in 2 senses — narrow and broad (LUSH, 1956; KEMPTHORNE, 1957). The 2 terms are defined as follows:

$$H\ (\text{narrow-sense}) = \frac{\text{Additive genetic variance}}{\text{Total variance}}$$

$$H\ (\text{broad-sense}) = \frac{\text{Total genetic variance}}{\text{Total variance}}$$

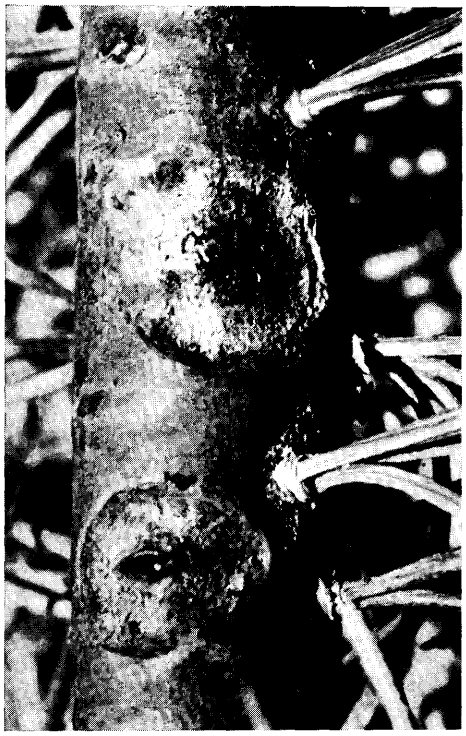

Figure 2. — Lesions in the vicinity of incipient cankers at needle bundle bases. The reaction appears to be associated with bark hypersensitivity.

Figure 3. — More extensive wound cambia in the region where an established bark canker was "corked out".

There are many versions of these heritability formulae, the differences among them being related to the type of mating system involved or to the genetic history of the organisms being investigated rather than to discrepancies in genetic or statistical theory. We chose as our model the formulae given by GRAFIUS (1952) and GRAFIUS, NELSON, and DIRKS (1952) because they apply to experiments in which sexual progenies, resulting from crosses between parents whose genetic constitution is unknown, are used, as is true with most forest tree progenies. The formulae are:

$$H\ (\text{narrow-sense}) = \frac{\sigma^2_f + \sigma^2_m}{\sigma^2 + \sigma^2_f + \sigma^2_m + \sigma^2_{fm}}$$

$$H\ (\text{broad-sense}) = \frac{\sigma^2_f + \sigma^2_m + \sigma^2_{fm}}{\sigma^2 + \sigma^2_f + \sigma^2_m + \sigma^2_{fm}}$$

In this experiment, quantitative data on variation in rust infection was obtained by computing percentage of survival in 10-seedling rows of full sibs. The basic replicate is thus a row of 10 full sibs, with the results that about 9/10 of the additively genetic variance due to differences in individual full sibs within rows (probably constituting about one-half of the total additively genetic variance) is lost in the averaging process. Furthermore, the statistics called heritabilities herein are not heritabilities in the strictest sense — rather, they are heritabilities of the differences which exist between the average performance of 10 full sibs within replicates.

In view of the abnormalities associated with data of this particular experiment, authors and reviewers agree that use of the basic heritability formulae above will result in underestimates of heritability. Neither numerators nor denominators contain all of the genetic variance present in the experimental materials, and where selection is to be on the basis of individual full sibs, the error term of the denominators remains disproportionately large. No practical means of adjusting σ^2_f σ^2_m, and σ^2_{fm} variance components to include the missing variance has been found. Instead, the method of GRAFIUS and WIEBE (1959), was used, wherein selection is based on strain means (in this case progeny means) and where the denominator is reduced by dividing the error variance component (σ^2) by the number of replicates (r). The basic heritability formulae then appeared as shown below:

$$\text{H (narrow-sense)} = \frac{\sigma^2_f + \sigma^2_m}{\sigma^2/_r + \sigma^2_f + \sigma^2_m + \sigma^2_{fm}}$$

$$\text{H (broad-sense)} = \frac{\sigma^2_f + \sigma^2_m + \sigma^2_{fm}}{\sigma^2/_r + \sigma^2_f + \sigma^2_m + \sigma^2_{fm}}$$

Calculation of genetic gain per generation of breeding is based on the formula, Genetic Gain (S) = Narrow-Sense Heritability × Selection Differential (ROBINSON, COMSTOCK, and HARVEY, 1949; LUSH, 1956).

Results and Discussion

Superiority of Progenies of the Resistant Phenotypes

In evaluating summarized raw data from the progeny tests, we found it helpful to array control- and wind-pollinated progenies of all 21 resistant phenotypes by parental lines *(table 1)*. Percentages of surviving seedlings in each progeny from a 2-parent mating appear twice in the array, once opposite "line" parent and once under "other" parent — regardless of whether male or female. Survivors among controls were considered to be mostly "escapes" (plants surviving merely by virtue of the fact that they escaped inoculation). It must be stressed that the recording of progenies under both line and other parents amounts to artificially doubling the data and degrees of freedom. Data in this form cannot be used for variance or heritability analyses. They do, however, serve to confirm the superiority of parents like numbers 17, 19, 22, and 58, and the inferiority of others like numbers 16, 29, and 30. The uniformity of progeny values within lines suggests that heritability of rust resistance may be fairly high, and the arrays aid in visualizing the more productive types of heritability analyses.

Heritability analyses were made only on the unadjusted or basic scorings like those for the progenies of 18 parents shown in *table 2*. That table includes data from all matings between trees that were represented by 2 or more matings, and in which each mating was represented by all 9 10-seedling row plots or replicates. Actually, in 1 progeny 1 row plot was missing; hence, all matings except 1 were represented by all 9 replicates. The value for this 1 missing plot was estimated by the method outlined in SNEDECOR (1946), and the numbers of degrees of freedom in all ana-

Table 1. — Percentages of surviving seedlings recovered in progenies of 21 parents, arrayed by parental lines¹)

Line parent	Other parent																						Number progs.³)	Line mean³)	Ranking
	1	10	15	16	17	18	19	20	21	22	23	24	25	27	28	29	30	37	38	39	58	OP²)			
1			8	7			18	5		35						3	9						7	12.1	
10							16						8										2	12.0	
15	8												18				14					6	3	13.3	
16	7						7	6	5		12		4				3				15	9	8	7.4	
17							37*		34	36*	23		25				24	33			49*	20	8	32.6	1
18							16						12				14				13	5	4	13.8	
19	18	16		7	37	16	34	23	40	30*					22	5	16		18	24	28*	7	14	21.4	6
20	5			6			23			40		25		5			6			4		3	8	14.2	
21				5	34		40						6				5	1			16	14	7	15.3	
22	35				36		30	40					22			6	17				34*	6	8	27.5	3
23				12	23																	13	2	17.5	8
24								25												25	38	7	3	29.3	2
25		8	18	4	25	12			6	22				*Selfs*			19⁴)				35	11	9	16.6	
27								5														2	1	5.0	
28							22															0	1	22.0	5
29	3						5			6											24		4	9.5	
30	9		14	3	24	14	16	6	5	17			19⁴)					8			21	6	12	13.0	
37					33				1								8				23		4	16.2	9
38							18																1	18.0	7
39							24	4				25								15	11		4	16.0	10
58			15		49	13	28		16	34		38	35			24	21	23		11	46	14	12	25.6	4
OP			6	9	20	5	7	3	14	6	13	7	11	2	0		6				14	5.3⁵)	15	8.2	

¹) Average of 9 replicates, together totaling 81 seedlings.

²) Wind-pollinated or 1-parent progenies.

³) Excluding all self- or open-pollinated progenies.

⁴) Reciprocal progenies, percentages averaged.

⁵) Average survival in 5 presumably nonresistant control progenies (total 400 seedlings) was 5.3 percent, considered mostly "escapes".

Table 2. — Mean percentages of surviving white pine seedlings in the 53 control-pollinated progenies used in heritability analyses[1]

Female parent	Male parent												
	1	10	16	17	18	21	22	24	25	29	30	37	39
	Percent												
15	8	—	—	—	—	—	—	—	18	—	14	—	—
16	7	—	—	—	—	5	—	—	4	—	3	—	—
17	—	—	—	—	—	—	—	—	25	—	24	—	—
19	18	16	7	37	16	40	30	—	—	5	16	—	24
20	5	—	6	—	—	—	40	25	—	—	6	—	4
21	—	—	—	34	—	—	—	—	—	—	—	1	—
22	35	—	—	36	—	—	—	—	22	6	17	—	—
23	—	—	12	23	—	—	—	—	—	—	—	—	—
25	—	8	—	—	12	6	—	—	—	—	17[2]	—	—
30	9	—	—	—	—	5	—	—	21[2]	—	—	8	—
58	—	—	15	49	13	16	34	38	35	24	21	23	11

[1] Eighteen different parents, each represented by 2 or more control-pollinated progenies, complete with 9 row plot replicates, are included.

[2] Reciprocal progenies, difference nonsignificant.

Table 3. — Percentages of survivors among progenies of selected and average phenotypes, and significance of the differences between and within types of mating

Type of mating	Progenies tested	Average survival	Gain in resistance
	Number	*Percent*	*Percent*
1. Average phenotype × wind (controls)	5	5.3[1]	
2. Selected phenotype × wind	13	9.3[1]	4.0
3. Selected phenotype × selected phenotype	47	17.9[2]	12.6
4. Selected phenotype progeny tested, then reselected on basis of best progenies regardless of type of combining ability × same	26	32.1[3]	26.8
5. Selected phenotype progeny tested, then reselected for highest level of general combining abitity for rust restistance × same	6	35.3[1]	30.0

Source of variation	Progenies	Degrees of freedom	Sum squares	Mean squares	F value
	Number	*Number*	*Number*	*Number*	*Number*
Types of mating (1—5 above)					
Between types	4	3	23575	7858	12.6[4][5]
Within types	71	67	41762	623	3.6[4]
Replicate	9	8	18039	2255	13.0[4]
Error		558	96400	173	
Total	639	636[6]	179776		

[1] Differences between progeny means making up this type of mating mean not significant at the 5 percent level.

[2] Differences between progeny means making up this type of mating mean significant at the 1 percent level.

[3] Mean survival estimated from 2-parent progeny test results (*table 1*) including all progenies made (26 out of a possible 45) between the 10 parents producing the best progenies (parents 1, 17, 19, 20, 21, 22, 24, 25, 37, and 58).

[4] Significant at the 1 percent level.

[5] F computed from within type/between type ratio.

[6] 638 degrees of freedom minus 2 missing replicates.

lyses including that mating were appropriately reduced by 1.

Effectiveness of phenotypic selection, and of selection after 1 generation of progeny testing, is demonstrated in *table 3*. Indicated differences between types of mating are highly significant. The gain to be realized through phenotypic selection — i. e. through collection of wind-pollinated seed from rust-free parents vs. that to be realized by collection of seed from ordinary nonresistant trees — appears to be small (4.0 percent). Likewise, the gain from crossing untested phenotypes — i. e. similar to collection of seed in orchards composed of grafts of parents that are not progeny tested — is also small (12.6 percent). But gains possible through genotypic selection — i. e. through collection of seed in grafted orchards, scionwood coming from parents which have been reselected for specific or general combining ability on the basis of progeny tests — are considerable (26.8 percent to 30.0 percent). Gains in orchards composed of surviving individuals from within tested progenies might be even greater, and possibly might be less expensive to attain.

Depending on the magnitude of the correlation of juvenile-tree vs. mature-tree resistance, and upon the relation between the observed, experimental level vs. the practical or field level of resistance, it appears that establishment of orchards with grafts of progeny-tested parents may be feasible in the near future. Such orchards could be the means of producing at least partially resistant planting stock for meeting immediate planting needs.

It is interesting, if perhaps fruitless to speculate on the low gain experienced when using 1-parent (wind-pollinated) test materials. It now appears that tests on wind-pollinated progenies may have to be 3 to 4 times as large as those using control-pollinated progenies. This is partly due to the greater variability of the wind-pollinated seedlings, but

Table 4. — Summary of correlations: Percentage of survival in mean half sib, 2-parent progenies vs. that in wind, self, random 2-parent, and test tree progenies of the same parent

Mean half sib 2-parent progeny versus:	Parents	2-parent half sib progenies/parent	Degrees of freedom	Correlation coeff.	Significance
	Number	*Average number*	*Number*	*r*	*Level*
A. Wind (1-parent) progenies	9	9.1	7	.516	N.S.
B. Self progenies	5	10.2	3	.798	N.S.
C. Random 2-parent progenies	9	6.8	59	.507	1%
D. 2-parent progenies all × same test tree	9	6.8	50	.668	1%
E. 2-parent progenies all × same 2 test trees	10	7.8	14	.817	1%
F. 2-parent progenies all × same 3 test trees	7	6.8	5	.910	1%

also possibly to accidents of pollination in a given year. Wind-pollinated tests may have to be repeated, using seed produced in more than 1 year. Also, because of the possibility of selfing, especially in isolated and/or self-compatible trees, wind-pollinated progenies may never give a reliable test.

Relative reliability of the various test materials may be brought out in another manner, as in *table 4*. There it is seen that survival in the average wind-pollinated progeny (wind half sibs) was not significantly correlated with mean survival in all 2-parent, half sib progenies of the same parents ($r = .516$, nonsignificant). On the other hand, survival in any 2-parent progeny was significantly, if weakly, correlated with that in the mean half sib progeny of the same parents ($r = .507$, significant at the 1 percent level of probability). Furthermore, when either 1, 2, or 3 of the same test trees were used to produce 2-parent progenies, then survival in the average test tree progeny(ies) was significantly, and often highly, correlated with that of the mean half sib progeny of the same parents. Obviously, the sensitivity of the test was increased by controlled pollination, more so by test tree crossing.

We should be neither disappointed nor complacent about the level of resistance attained in 1 generation of breeding. As expected, we did not secure 100-percent resistant stock, and appreciable increases in the level of resistance will come only from continued selection and breeding in subsequent generations. Progress toward that goal will depend largely on the heritability of rust resistance, on the intensity of selection we are able to exert prior to production of each successive generation, and on shortening the time required for progeny test procedures. These factors are considered in detail below.

Heritability of Rust Resistance

At the time the original crosses were made in 1950, we realized that it would be desirable to conduct a diallel crossing program in which each phenotype was crossed with every other phenotype. With only the 18 parents listed in *table 2*, such a program would have involved $\frac{18 \times 17}{2} = 153$ different matings. This was beyond the flower producing capacity of the trees in a single year. Instead, an irregular mating pattern was followed, in which the choice of combinations was governed mostly by non-genetic considerations, i. e. sometimes by pollen availability in relation to time of female flower receptivity, but principally by number of female flowers per tree.

It was realized that because of the irregular mating pattern followed, any 1 heritability analysis of data in *table 2* probably might be unreliable. Hence, 4 different heritability analyses of these data were made. One of these analyses included those portions of the data obtained from mating 2 female parents with 8 male parents in all possible combinations (i. e. 16 progenies). Two of the analyses included those portions of the data obtained from mating 2 trees with 8 other trees (16 progenies) and 3 trees with 5 other trees (15 progenies) in all possible combinations, but with the individual parents being used as males in some combinations and as females in others.

Little can be said in respect to the results of these first 3 heritability analyses, other than that they were inconclusive. Test materials were scanty, including only 15 to 16 progenies, and thus the tests were not conducted at a level sufficient to permit accurate separation of the components of genetic variance. While total variances were quite similar, the female, male, and female × male interaction components varied widely in the 3 different analyses, apparently depending on the particular females and males employed.

The fourth analysis was believed more conclusive, since it included all 53 control-pollinated progenies listed in *table 2*, and since variance components were quite similar to those computed in 1 of the 3 analyses described above. It is presented in *table 5*.

Some Practical Aspects of the Type of Selection Employed

We feel that a point has been reached where it will be profitable to draw some tentative conclusions regarding the type of selection likely to be most rewarding for continued, early-generation breeding work. Some of the factors that must be considered in the choice of breeding schemes are: (1) minimum number of parents necessary for prevention of vigor deterioration through inbreeding, (2) relative efficiency of selection for general vs. specific combining ability in the early generation testing, and (3) possibilities of tapping the breeding scheme at any point for exploitation of short-term gains.

Good possibilities exist for exercising selection against potentially self-fertile parents (Bingham and Squillace, 1955; Langner, 1956) or against parents exhibiting little or no selective fertilization toward prevention of self-fertilization under conditions of mixed pollination (Squillace and Bingham, 1958). Also, there is a good possibility of reducing the extent of selfing in seed orchards by merely positioning ramets within clones so that none occur in close proximity (Langner, 1953). Nevertheless, until more is known about the extent and effects of inbreeding, it will be best to assume random pollination and fertilization in prospective orchards. Under these conditions, the increase in homozygosity due to inbreeding, over a period of 4 generations when using only 10, 5, 4, 3, 2, or 1 clones, is demonstrated by the corresponding inbreeding coefficients as .18, .34, .42, .52, .68, or .94 (1.00 equals complete inbreeding). Stern (1959) suggests a minimum of 20 to 30 clones under random pollination. Obviously, we must include as many clones as possible in future seed orchards. Possible 25 to 30 would be a safe minimum.

In respect to genetic gains in resistance through continued breeding, it is probably true that the greatest long-term gains will come from increasing selection differential by eventual selection for specific combining ability. Probably it is equally true that the most practical method, assuring easy exploitation of current gains while making rapid progress toward substantially increased resistance, will be through early-generation selection for general combining ability. These beliefs are borne out by the findings of Sprague and Tatum (1942), as well as by the data assembled in *Tables 1* and *6*. In *table 1* the consistently good performance of progenies of parents 17, 19, 22, and 58, each represented by 8 to 14 progenies, is clearcut. It is also particularly noteworthy that performance of progenies from crossings between these 4 parents is especially good. *Table 1* also demonstrates some notable examples of what appears to be specific combining ability, i. e. in progenies like 19 × 21 and 20 × 22, where performance is well above the level to be expected on the basis of general combining ability of parents 21 or 20. Even where both parents have demonstrated general combining ability for fairly high-level resistance, we find some progenies like 58 × 17, where the level of resistance appears to exceed

Table 5. — Variance and heritability analyses of arcsine-transformed percentages of survivors in control-pollinated matings of *table 2*, above[1])

Analysis of Variance

Source of variation	Degrees of freedom	Mean square	Expectation mean square	Variance component
Progeny				
Female parent (F)	10	1,771.00[2])	$\sigma^2 + 9\sigma^2_{fm} + 9 \times 3.5022 \times \sigma^2_f$	$\sigma^2_f = 43.59$
Male parent (M)	12	2,069.43[2])	$\sigma^2 + 9\sigma^2_{fm} + 9 \times 3.4457 \times \sigma^2_m$	$\sigma^2_m = 53.64$
Total additively genetic variance				97.23
Female x male (FM)	30	396.97[2])	$\sigma^2 + 9\sigma^2_{fm}$	$\sigma^2_{fm} = 25.58$
Total genetic variance				122.81
Replicate (R)	8	2,839.96[2])		
Error (FMR)	416	166.74	σ^2	$\sigma^2 = 166.74$[3])
Total	476			

Heritability Estimates

$$H\ (\text{broad-sense}) = \frac{\sigma^2_f + \sigma^2_m + \sigma^2_{fm}}{\sigma^2/_r + \sigma^2_f + \sigma^2_m + \sigma^2_{fm}} = \frac{122.81}{141.34} = 0.869^{3)}$$

$$H\ (\text{narrow-sense}) = \frac{\sigma^2_f + \sigma^2_m}{\sigma^2/_r + \sigma^2_f + \sigma^2_m + \sigma^2_{fm}} = \frac{97.23}{141.34} = 0.688^{3)}$$

[1]) In the estimation of variance components the numbers of female and male parents were the harmonic means ($k_f = 3.4457$ and $k_m = 3.5022$) of the numbers of matings entered into by the males and females, respectively. There were 9 replicates, each a 10-seedling row of full sibs.

[2]) Significant at the 1 percent level.

[3]) See discussion page 35—36.

that expected on the basis of general combining ability of both parents.

Table 6 compares the best progenies from crossings among the 23 parents in *table 1*. Arrayed on the left are the 6 progenies of parents 17, 19, 22, and 58, (indicated by asterisks in *table 1*) or the type of progenies that would be selected if breeding were to be channeled primarily toward general combining ability. Arrayed on the right are the 6 best progenies of the type which might be selected if breeding were to be directed toward the greatest immediate gain through both general and specific combining ability. Where selection is directed more toward specific combining ability, as in the 6 progenies on the right, the selection differential is increased by about 5 percent.

Obviously, with heritabilities near 0.65, the 5-percent advantage accruing to selection for specific combining ability is not large enough to warrant establishment of 6 separate seed orchards required for production of 34.7-percent resistant seed — at least not when a single orchard should produce the 30.0-percent resistant seed from parents like 17, 19, 22, and 58. While it is true that large gains due to selection for specific combining ability are possible, they usually come after several generations of selection for general combining ability (SPRAGUE and TATUM, 1942). Thus, as we approach an acceptable level of field resistance, a selection for specific combining ability might well be made, perhaps among progenies from a diallel cross, mating parents which have already undergone a few generations of selection for general combining ability.

Lastly, the breeding scheme that proposes early-generation selection for general combining ability may be tapped easily and at any point, permitting exploitation of interim gains in relatively uncomplicated seed orchards.

Calculation of Genetic Gain

Data and analyses covered thus far have practical value since they may be used to calculate the per-generation genetic gain to be expected from future breeding work.

Three points should be emphasized before undertaking estimates of genetic gain: (1) Calculated heritabilities are specific to the particular set of test conditions under which derived, and the genetic gains estimated therefrom apply only under the same conditions. (2) Estimated gains must be applied with caution to less well-replicated experiments, but are applicable to experiments of longer duration, provided that age of the test materials does not affect their resistance appreciably. (3) Practical use of indicated gains depends upon finding another 25 or more parents (possibly within each of 3 elevational zones) exhibiting a promising level of general combining ability for rust resistance.

As stated previously, genetic gain per generation (S) = narrow-sense heritability (H) × selection differential (s). Using the narrow-sense heritability calculated for all progenies as in *table 5* (0.688) and the selection differential

Table 6. — Percentage of survival in 6 progenies of 4 parents exhibiting general combining ability for high-level resistance vs. that in the 6 best progenies regardless of type of combining ability of the parents[1])

Progenies of 4 parents with demonstrated general combining ability		Six best progenies regardless of type of combining ability involved	
Progeny	*Percent survival*	*Progeny*	*Percent survival*
58×17	43	58×17	43
19×17	32	19×21	35
22×17	30	20×22	35
58×22	28	58×24	33
19×22	25	19×17	32
19×58	22	22×17	30
Averages (Sel. diff.)	30.0		34.7
Difference =		4.7[2])	

[1]) All percentages reduced 5.3 percent or by the average level of survival in 5 presumably nonresistant control lots.

[2]) Significant at the 5 percent level.

(26.8 percent increase in resistance over controls) shown in *table 3* for the best progenies of 10 different parents, progress per generation can be estimated at about 18 percent. Using the same narrow-sense heritability (0.688) and the selection differentials (30.0 and 34.7) calculated for the progenies of *table 6*, the gain per generation is estimated at 21 or 24 percent.

It should be emphasized here that gains in the second and successive generations cannot be predicted with absolute assurance at the present stage of our studies. There are genetic models in which all improvement is obtained in the first generation. On the other hand, successive gains have been made in the majority of short-lived organisms, a selection plateau beyond which further progress was negligible being reached only after many generations of breeding work. In respect to rates of gain, these rates may possibly be increased in 3 ways: (1) Heritability might be increased by producing and testing larger progenies in more nearly uniform environments, possibly employing a number of standard test crosses to appraise all parents. (2) The selection differential might be increased by discovering new and better parents and progenies. To date, fewer than 10 percent of the field selections have been completely tested. Chances are that even better parents and progenies will be found. (3) The "field" level of resistance may prove to be higher than the "experimental" level of resistance.

Summary

Progeny tests were started in 1952, using 61 control-pollinated and 15 wind-pollinated progenies of rust resistant western white pine phenotypes *(Pinus monticola* Dougl.*)*, along with 5 wind-pollinated progenies from normal, nonresistant phenotypes. Progenies were grown 2 years in a nursery at Spokane, Washington, where they were artificially inoculated with the white pine blister rust fungus *(Cronartium ribicola* Fischer*)*. They were then outplanted to await rust development, using 3 semi-permanent test plantings near Clarkia and Elk River, Idaho, and Saltese, Montana. Natural exposure to the rust occurred at the outplanting sites. In the fall of 1957, when seedlings were 6 years old, they were given a final scoring for the presence or absence of symptoms of the rust fungus.

Progenies of average phenotype × average phenotype, selected phenotype × wind, and selected phenotype × selected phenotype matings contained 5.3, 9.3, and 17.9 percent surviving seedlings, respectively. Differences among types of mating, and between progenies within the selected phenotype × selected phenotype mating type were highly significant. The mean control progeny (average phenotype × average phenotype) contained 5.3 percent survivors, probably mostly "escapes", despite the heavy, artificial inoculations employed. Meanwhile, the best 1-parent (selected phenotype × wind) progeny contained 20 percent survivors, and the best 2-parent (selected phenotype × selected phenotype) progeny 49 percent survivors.

Heritability of rust resistance was estimated by the method of expectation mean squares for 4 different groupings of related progenies. In the more reliable test, the narrow-sense heritability was 0.688 (68 percent), broad-sense heritability 0.869.

With the narrow-sense heritability value given above, and with selection differentials for different progeny groupings ranging from 26.8 to 34.7 percent, genetic gain per generation of breeding was calculated variously 18, 21, and 24 percent per generation. The gains are not assured, since the genetic constitution of the test materials remains little understood, but chances are that a selection plateau (beyond which progress by selection is nil) has not been reached.

Zusammenfassung

Titel der Arbeit: *Züchtung blasenrostresistenter Pinus monticola. II. Erste Ergebnisse von Nachkommenschaftsprüfungen einschließlich vorläufiger Berechnungen der Heritabilität und der „Züchtungsrate“.*

1952 wurden mit 61 Nachkommenschaften künstlicher Kreuzungen, mit 15 Nachkommenschaften frei abgeblühter Mutterbäume — beide von rostresistenten Phänotypen der *Pinus monticola* — sowie mit fünf Nachkommenschaften frei abgeblühter normaler, nicht resistenter Bäume Nachkommenschaftsprüfungen angelegt. Zwei Jahre verblieben die Sämlinge in einem Kamp in Spokane, Wash., wo sie mit dem Weymouthskiefern-Blasenrost *(Cronartium ribicola* Fischer*)* infiziert wurden. Später pflanzte man sie in drei „semipermanente“ Testflächen bei Clarkia und Elk River, Idaho, und bei Saltese, Montana. Dort standen sie unter natürlichen Befallsbedingungen des Rostes. Im Herbst 1957, im Alter 6, fand eine abschließende Bonitierung der Symptome des Rostbefalls statt.

Nachkommen einer Kreuzung zwischen durchschnittlichen, nicht selektierten Eltern enthielten 5,3% Überlebende, Nachkommen frei abgeblühter, selektierter Mutterbäume 9,3% und Nachkommen der Kombination „selektiert × selektiert“ 17,9% Überlebende. Differenzen zwischen den drei genannten Gruppen wie auch zwischen verschiedenen Nachkommenschaften des Typs „selektiert × selektiert“ waren hoch signifikant. Die mittlere Kontroll-Nachkommenschaft („durchschnittlicher Phänotyp × durchschnittlicher Phänotyp“) enthält 5,3% Überlebende, die trotz der heftigen künstlichen Infektionen wahrscheinlich in der Hauptsache zufällig ohne Befall blieben. Dagegen finden sich in der besten frei abgeblühten Nachkommenschaft („selektiert × Wind“) 20% und in der besten Kreuzungs-Nachkommenschaft (selektiert × selektiert) 49% Überlebende.

Die Heritabilität der Rostresistenz wurde aus den Variationskomponenten von vier verschiedenen Gruppierungen verwandter Nachkommenschaften ermittelt. In dem zuverlässigeren Test betrug die Heritabilität im engeren Sinne 0,688 (68%) und die Heritabilität im weiteren Sinne 0,869.

Mit dem genannten Wert der Heritabilität i. e. S. und den Selektionsdifferenzialen unterschiedlicher Nachkommenschaftsgruppierungen im Bereich von 26,8 bis 34,7% wurde der Selektionsfortschritt auf 18, 21 und 24% pro Generation geschätzt. Diese Werte sind nicht sicher, da die genetische Konstitution des Untersuchungsmaterials wenig bekannt ist. Aber es bestehen Aussichten, daß ein Selektionsplateau (jenseits dessen durch Selektion keine positiven Effekte mehr möglich sind) noch nicht erreicht wurde.

Résumé

Titre le l'article: *Amélioration de Pinus monticola pour la résistance à la rouille vésiculeuse. II. Premiers résultats des tests de descendance comprenant une estimation de l'héritabilité et du taux d'amélioration.*

Des tests de descendance furent installés en 1952; ils comprenaient 61 familles issues de pollinisation contrôlée et 15 issues de pollinisation libre, toutes descendant de *Pinus monticola*, présentant une résistance phénotypique à la rouille vésiculeuse. A titre de comparaison, 5 des-

cendances issues de pollinisation libre de phénotypes normalement non résistants étaient comprises dans l'expérience. Ces descendances furent cultivées pendant 2 ans en pépinière à Spokane, Washington; elles y subirent des inoculations artificielles avec l'agent de la rouille vésiculeuse (*Cronartium ribicola* Fischer). Elles furent ensuite plantées, pour observer le developpement de la rouille, dans trois dispositifs semi-permanents près de Clarkia et Elk River, Idaho, et Saltese, Montana. Dans ces stations, les plants étaient exposés à l'infection naturelle par la rouille. A l'automne 1957, les plants étant âgés de 6 ans, on procéda à un examen final pour détecter la présence ou l'absence des symptômes d'attaque de rouille.

Le tableau suivant donne les pourcentages de plants survivants pour divers types de croisement:

Phénotype moyen × par phénotype moyen: 5,3%,
Phénotype sélectionné (pollinisation libre): 9,3%,
Phénotype sélectionné × par phénotype sélectionné: 17,9%.

Les différences entre les types de croisement et entre les descendances à l'intérieur du troisième type de croisement (phénotype sélectionné × par phénotype sélectionné) étaient nettement significatives. La descendance témoin (phénotype moyen × par phénotype moyen) présentait 5,3% de survivants, la plupart ayant probablement échappé aux inoculations artificielles pourtant très sévères. La meilleure descendance uni-parentale (phénotype sélectionné — pollinisation libre) a 20% de survivants et la meilleure descendance bi-parentale (phénotype sélectionné × par phénotype sélectionné) 49%.

L'héritabilité de la résistance à la rouille fut estimée par la méthode des comparaisons de variances pour 4 groupements différents de descendances voisines. Dans l'expérience la plus précise, l'héritabilité au sens strict était de 0,688, l'héritabilité au sens large de 0,869.

En prenant la valeur de l'héritabilité au sens strict donnée ci-dessus avec des coefficients de sélection allant de 26,8 à 34,7% selon les groupes de descendance, l'amélioration génétique par génération fut estimée à 18, 21 et 24%. Ces gains ne seront pas acquis avec certitude puisque la constitution génétique du matériel d'essai reste peu connue, mais il y a des chances pour que l'on n'atteigne pas un palier dans la sélection au-delà duquel le progrès serait nul.

Literature Cited

Bingham, R. T., and Squillace, A. E.: Self-compatibility and effects of self-fertility in western white pine. Forest Sci. 1, 121—129 (1955). — Bingham, R. T., Squillace, A. E., and Duffield, J. W.: Breeding blister-rust-resistant western white pine. Jour. Forestry 51, 163—168 (1953). — Grafius, J. E.: A statistical model for estimating components of genetic variance in bulk yield tests of self pollinated small grains. S. Dak. Agric. Expt. Sta. Tech. Bull. 9, 13 pp. (1952). — Grafius, J. E., Nelson, W. L., and Dirks, V. A.: The heritability of yield in barley as measured by early generation bulked progenies. Agron. Jour. 44, 253—257 (1952). — Grafius, J. E., and Wiebe, G. A.: Expected genetic gain in yield in small grain. A geometrical interpretation. Agron. Jour. 51, 560—562 (1959). — Kempthorne, Oscar: An introduction to genetic statistics. John Wiley & Sons, New York, N. Y. 545 pp. (1957). — Langner, Wolfgang: Die Klonanordnung in Samenplantagen. Silvae Genetica 2, 119—121 (1953). — Langner, Wolfgang: Möglichkeiten der Saatgutgewinnung von züchterisch geprüften Einzelbäumen. Züchter 26, 276—278 (1956). — Lush, J. L.: Animal breeding plans, 3rd Ed. Iowa State Coll. Press, Ames, Iowa. 443 pp. (1956). — Robinson, H. F., Comstock, R. E., and Harvey, P. H.: Estimates of heritability and degree of dominance in corn. Agron. Jour. 41, 353—359 (1949). — Snedecor, George W.: Statistical methods, 4th Ed. Iowa State Coll. Press, Ames, Iowa. 485 pp. (1946). — Sprague, G. F., and Tatum, Lloyd A.: General vs. specific combining ability in single crosses of corn. Jour. Amer. Soc. Agron. 34, 923—932 (1942). — Squillace, A. E., and Bingham, R. T.: Selective fertilization in *Pinus monticola* Dougl. I. Preliminary results. Silvae Genetica 7, 188—196 (1958). — Stern, K.: Der Inzuchtgrad in Nachkommenschaften von Samenplantagen. Silvae Genetica 8, 37—42 (1959). — Struckmeyer, Esther, B., and Riker, A. J.: Wound-periderm formation in white pine trees resistent to blister rust. Phytopathology 41, 276—281 (1951). — Syrach Larsen, Carl: Estimation of the genotype in forest trees. Denmark Roy. Vet. & Agric. Coll. Yrbk. 1947, 87—128 (1947). — Toda, Ryookiti: Variation and heritability of some quantitative characters in *Cryptomeria*. Silvae Genetica 7, 87—93 (1958). — Toda, Ryookiti: The heritability of tree height and stem girth in *Cryptomeria* through sexual reproduction. Bull. Jap. Gov't. For. Expt. Sta. 112, 33—41 (1959).

Les vieux pins laricio greffés de la forêt de Fontainebleau

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Le greffage des arbres forestiers est devenu depuis une vingtaine d'années un des outils essentiels des techniques d'amélioration. Les procédés de greffage, en serre ou à l'extérieur, ont fait l'objet de nombreuses études théoriques et pratiques, qui ont permis de préciser les conditions optima pour la réussite.

Le greffage a été utilisé depuis des siècles par les horticulteurs pour la multiplication des formes ornementales des espèces forestières. Les techniques utilisées actuellement dans les stations d'amélioration sont dérivées directement des procédés traditionnels. On sait moins que le greffage des arbres forestiers a été pratiqué en France, il y a 120 ans, sur une grande échelle, et dans un but qui ne relevait pas de l'horticulture, mais qui était somme toute assez proche de celui que nous poursuivons: la production en masse de graines de bonne qualité génétique.

Les auteurs de ce travail, qu'on peut considérer comme des précurseurs de l'idée des vergers à graines, sont deux forestiers de Fontainebleau, le baron de Larminat et Marrier de Boisdhyver, respectivement Conservateur et Inspecteur des forêts de la Couronne, qui exercèrent leur activité dans la forêt de Fontainebleau dans les années 1820—1850.

Cette forêt, située à 50 km au sud de Paris, couvre environ 20 000 ha. Elle porte, sur ses meilleurs sols, des peuplements naturels de chêne rouvre; les plus mauvaises parties, sables et grès très pauvres, ont été partiellement enrésinées depuis le XVIIIe siècle surtout en pin sylvestre originaire des provinces baltes. Au début du XIXe siècle, ces enrésinements se poursuivirent, avec des pins sylvestres originaires de la forêt de Haguenau, et d'autres pins, notamment weymouth et laricio de Corse. De Larminat