Breeding Blister Rust Resistant Western White Pine

IV. Mixed-Pollen Crosses for Appraisal of General Combining Ability

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(Received for publication July 4, 1967)

In the Forest Service program for blister rust resistance improvement, now being carried out at Moscow, Idaho, we have been testing progenies of phenotypically resistant (rust-free) western white pine plus trees, or "candidate" trees from heavily infected natural stands. The tests are designed to appraise parental combining ability, or breeding value. We are attempting to secure a base in parent trees that will cross among themselves in all directions in seed orchards to produce highly resistant offspring; that is, we want parents that display high general combining ability for resistance and therefore have high breeding value when used in orchards (BINGHAM 1966, BINGHAM *et al.* 1953 and 1960).

To avoid the extraneous variation in resistance that may arise from irregularities of wind pollination – eibher from selfing or outcrossing – we have used control-pollinated progenies. In our present "standard" test, individual crosses are made with each of four "tester" trees. While this multicross test provides fairly reliable information on which to base selection, it is quite costly. Over a 10-year period during which 360 candidates have been tested, and despite the high efficiency of an experienced work force, cost per candidate tree averaged about $ 600.

In future work we propose the testing of another 2,500 or more candidates. Naturally, we are seeking less expensive methods of pollination and testing, as are others who are using or proposing to use multicross test methods in their work with blister rust resistance (Forest Service Regions 5, 6, and 9), fusiform rust resistance (Southeast Forest Experiment Station, North Carolina State College, University of Florida), and Scotch pine plus tree testing (Swedish Association for Forest Tree Breeding).

One promising method for reducing costs of crossing is substitution of a single mixed-pollen cross for the several crosses. Use of this method with trees was suggested by GODDARD, PETERS, and STRICKLAND (1962), but as far as is known it remains untested. Since controlled mixed-pollen crosses seemed likely to provide fairly reliable estimates of combining ability, we set out. to test this hypothesis, using the methods described below.

Materials and Methods

Mating scheme. – Sixteen blister rust resistant western white pine plus trees from five heavily infected natural stands in northern Idaho were utilized as mother trees in crosses made in the manner shown in ***Table*** 1. Ripe pollens were collected from 10 tester trees, then extracted and used within a few days in making the series of six controlled crosses. Pollen germination tests were not attempted, because when freshly collected pollens are used, results of these tests seem to bear little relation to seed set in western white pine. Eleven plus trees were crossed in 1961, and five more were crossed in 1962.

Experimental design. – Mature cones that developed from the pollinations were collected, and the seed was extracted,

Table 1. – The series of 6 crosses made on each of 16 plus trees

Cross no.	Pollination	Term for type of cross
1	Plus tree X individual ♂ tester tree	Standard cross (Average of 4 individual crosses)
2	Same plus tree X 2nd individual tester	
3	Same plus tree X 3rd individual tester	
4	Same plus tree X 4th individual tester	
5	Same plus tree X equal-volume mix of pollens of the 4 testers above	4-pollen cross
6	Same plus tree X equal-volume mix of pollens of 4 testers above, plus pollens of 6 other testers	10-pollen cross

cleane$ and counted. The sound seed was sown in September and October of the years following pollinations, that is, in 1962 and 1963. Seed was sown in 10 randomized blocks, each block containing a single replicate (3" X 24", 16-seed plot) of each progeny. Thus in 1962 there were 66 progenies (representing 6 crosses X 11 mother trees), and in 1963 there were 30 progenies (6 crosses X 5 mother trees). Six ordinary and presumably nonresistant control seed lots were sown in the same design in the 1962 test; 8 were sown in the 1963 test.

Inoculation and seedling examination. – Seedling progenies were twice artificially inoculated with the blister rust fungus, first when the seedlings were 1 year old (and extremely susceptible), and again when they were 2 years old. Inoculation procedures, utilizing rust-infected wild currant bushes, 'have been described previously (BINGHAM *et al.* 1953, BINGHAM 1966). During inoculation, pine-infecting blister rust sporidia were trapped on vaseline-coated microscope slides so that the average spore cast, and the variation therein, could be estimated. Spore-cast slides were placed at seedling level and spaced uniformly throughout the inoculation chamber. They continued to accumulate sporidia until the chambers were dismantled after about 72 hours.

Rust examinations to determine the presence of active blister rust bark lesions were made in August of 1964 (1962 test only) and 1965. Data considered here come from the 1965 examination, made 2 years after initial inoculation of the 1962-sown seedlings, but only one year after initial inoculation of the 1963-sown seedlings. Resistance was expressed as the percent of healthy seedlings remaining in each plot at this final examination.

Analysis of ***results.*** – Because basic data were binomial in nature (percents of healthy seedlings per plot) they were transformed to angles equal to the arcsins of the square root of percents healthy (BARTLETT 1947 and SNEDECOR 1946, pp. 431–452).

Also, because the numbers of seedlings per plot were often small (maximum of 16), adjustments to prevent wide fluctuations in percent, and thus stabilize variance, were made (BARTLETT 1936). Thus when the number of healthy seedlings equaled the total number of seedlings in the plot, the percent of healthy seedlings was adjusted using the following formula:

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percent healthy =

$$1 - \left| \frac{1}{4 \times \text{total number of seedlings in the plot}} \right| \times 100,$$

and when the number of healthy seedlings in the plot was zero, by the formula:

percent healthy =

$$\left| \frac{1}{4 \times \text{total number of seedlings in the plot}} \right| \times 100.$$

These basic data, along with seed yield and spore-cast data, were entered into a series of analyses attempting to define variability between the two types of mixed-pollen crosses, and their consistency, acceptability, and practical usefulness when substituted for the corresponding standard crosses, as follows:

1. Possible selective fertilization or other pollen effects were evaluated by comparison of seed yield, and of heterogeneity in mean percent of healthy seedlings, observed in the 4- vs. the 10-pollen-mix crosses.
2. Mean percents of healthy seedlings observed in the two mixed-pollen crosses were correlated with those observed in the corresponding standard crosses.
3. Accuracy and reliability of the two mixed-pollen crosses was tested, assuming that the standard cross was accurate, and considering bias (probably associated with pollen effects) as well as other variability of the mixed-pollen crosses.
4. Relative efficiency, or sensitivity, of the three types of crosses was compared, using ratios of variances associated with plus trees and error; then observed ratios were used to estimate number of sample plots (replicates) required for securing accuracy in mixed-pollen crosses equal to that of the standard crosses. Probable influence of patchy inoculations on magnitude of error variance was considered.

Comparisons were also made of the results of practical selection when mixed-pollen crosses were substituted for the presumably more accurate but more costly standard crosses.

Results and Discussion

Sound and hollow seed yields. — Seed yields for the six crosses for the 2 years are shown in *Table 2.*

The variation in seed yield among the four individual crosses comprising the standard cross suggests that individual testers, or their pollens, varied in their ability to effect seed set. Note that in 1961 cross 2 and in 1962 cross 1 produced both the highest hollow seed yields and the lowest sound seed yields and thus the highest proportions of hollow to total seed. If pollens of these two testers (or of other testers in the 10-pollen mix) had similarly low seed-setting ability, then these low-grade pollens may not have fertilized the expected proportion (25 or 10 percent) of ovules. Mean seed yield data did not show distinctive differences between the standard cross and either of the mixed-pollen crosses, or between the 2 mixed-pollen crosses. However, chi-square analyses of heterogeneity made on the seedling data supported the belief that fresh pollens were somehow interacting in mixture, or, more likely, were of differential germinability or potency.

Table 2. — Seed yields for the four individual crosses of the standard cross, and for the mixed-pollen crosses.

Cross no.	Average number seeds per cone					
	1961 crosses (11 mother trees)			1962 crosses (5 mother trees)		
	Hollow	Sound	Hollow/total	Hollow	Sound	Hollow/total
			(Percent)			(Percent)
1	15.4	90.4	15	17.4	60.4	22
2	42.1	73.7	36	10.4	66.5	14
3	29.1	93.7	24	12.0	71.5	14
4	15.2	82.3	16	12.2	75.1	14
1—4 average	25.4	85.0	22.8	13.0	68.4	16.0
5	16.9	84.4	17	14.2	67.9	17
6	21.4	93.9	19	13.9	67.3	17

Theoretically, significant effects of differences in individual pollens would be far more likely in the 4-pollen mix crosses than in the 10-pollen mix crosses, since each pollen makes up 25 percent of the 4-pollen mix, but only 10 percent of the 10-pollen mix. Again, the heterogeneity chi-square analyses of seedling data supported this hypothesis. Probably, variation in potency of males is to be expected. It is discussed here only to emphasize the advantage of including many pollens in the mix, and to point out that it is a possible source of variation between results of the mixed-pollen and standard crosses.

Effectiveness and uniformity of inoculations. — Artificial inoculations were highly effective, culminating in generally heavy infection throughout the experimental seedbeds. Intensity of exposure of test seedlings to the rust was indicated by the numbers of sporidia per square millimeter, as trapped on spore-cast slides. *Table 3* gives results of the spore trapping.

Inoculations in which the average seedling probably intercepted 2,000+ to 10,000+ sporidia — a third or more known to be viable — certainly should have been effective. But even with the massive exposure, variation in the spore cast shown on individual slides indicated the high probability that individual seedlings and plots received greatly different degrees of exposure.

Overall effectiveness of the inoculations was measured by subsequent development of the blister rust disease on the ordinary, nonresistant control seedlings. In the six control lots (60 plots) sown in 1962, only 3.0 percent (S. E. ± 0.7 percent) of 744 seedlings remained healthy through

Table 3. — Intensity and variation in sporidial cast under artificial inoculation.

Inoculation	Number of slides	Sporidia			
		Observed germinating[1] (average)	Total (average)	Range in total	Estimated total per seedling[2]
		Number/sq. mm.			
1963:					
Seedbeds sown in 1962	18	2.7	6.1	0—11	2,900
1964:					
Seedbeds sown in 1962	18	1.7	3.9	0—8	7,800
Total:					
1962 seedbeds	36	4.4	10.0	—	10,700
Seedbeds sown in 1963	24	2.1	4.8	0—15	2,280

[1]) Germ tubes longer than spores.

[2]) Based on the mean 1-year-old seedling having 425 square millimeters of upper leaf surface (on 38.5, 12.5 mm. long by 1 mm. wide primary needles), and on the mean 2-year-old seedling having 2,000 square millimeters of upper leaf surface (on 50, 40 mm. long by 1 mm. wide secondary needles).

1965, or 2 years after first inoculation. In the eight lots (80 plots) sown in 1963, complementary results were 8.1 percent (± 1.0 percent) of 1,072 seedlings, one year after first inoculation.

Progenies of rust-free plus trees also became heavily infected. In the 1962-sown experiment, by 1965 the average 16-seed plot contained 12.3 established seedlings, of which 11.3 were cankered. Complementary results for the 1963-sown experiment were 12.3 seedlings, 10.8 of which were infected.

Despite overall effectiveness of inoculation, percentages of infection in plots varied between blocks, apparently due to (1) localized variation in intensity of inoculation, and (2) the binomial (percentage) nature of the response — especially where row-plots contained small numbers of seedlings.

Variation in level of infection due to patchy inoculation was foreshadowed in the relatively wide range of sporidia per square millimeter found on the spore-cast slides. It is believed, however, that most of the variation was not due to microclimatic variations in the inoculation chambers but, instead, arose from two other sources: the small size of the individual row-plot replicates (3" × 24"), and the nature of the inoculum (the inoculum consisted of "families" of rust-infected wild currant leaves still attached on bushes that were often quite large and that varied widely in degree of infection, thus varying in the amount of inoculum they could produce). Given these two conditions, it is evident that one or several of the small plots might be inoculated principally from a single very lightly or heavily infected bush that happened to be placed above them.

The variation in infection, like the overall effectiveness of the inoculation, was measurable by the percentage of healthy seedlings in the 140 control plots. Although control seedlings, on the average, became about 95 percent infected, 18 of the 140 control plots contained over 15 percent healthy plants and averaged 28 percent healthy plants. Obviously, patchy inoculation increased error and reduced the reliability of results. It would seem that if more uniform dispersal of sporidia were achieved, as by means of randomly placed, detached currant leaves, randomly placed smaller bushes, or possibly movement of spore-laden air during periods of maximum spore cast, much of this variation would be eliminated.

Reliability of mixed-pollen crosses. — Percentages of healthy seedlings found in 1965 in the standard or 4-cross progenies are compared in *Table 4* with those found in the two types of mixed-pollen cross progenies (columns 3, 6, and 10).

Product-moment correlation analysis of the adjusted and transformed data showed that results of both the 4-pollen and 10-pollen crosses were significantly related to the results of the standard cross (pooled r with 13 $d.f.$ =0.765 (4-pollen cross) and 0.918 (10-pollen cross), both significant at the 1% level of probability). The correlation, however, provided no measure for judging accuracy of mixed-pollen results for individual trees, and thus no basis for developing reliability criteria for use in the selection or culling of individual plus trees.

Proceeding toward reliability criteria, we reasoned that error variance, even in the standard cross, is likely to be large, for reasons discussed above. With the probably less

Table 4. — Adjusted[1]) percent of healthy seedlings, and transformed[2]) percent differences between standard crosses and mixed-pollen crosses.

Plus tree	Standard crosses			4-pollen mix crosses				10-pollen mix crosses			
	Number of trees tested	Healthy seedlings (expected result)		Number of trees tested	Healthy seedlings (observed result)		Difference observed-expected	Number of trees tested	Healthy seedlings (observed result)		Difference observed-expected
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
		%	Transf. %		%	Transf. %			%	Transf. %	
						1962 test					
61	511	10.5	18.91	120	13.0	21.13	+ 2.22	130	15.9	23.50	+ 4.59
69	439	11.8	20.09	95	13.4	21.47	+ 1.38	120	14.9	22.71	+ 2.62
70	476	7.6	16.00	113	15.2	22.95	+ 6.95	130	7.1	15.45	— 0.55
224	585	4.9	12.79	149	2.2	8.53	— 4.26	147	4.4	12.11	+ 0.68
257	591	4.3	11.97	146	2.8	9.63	— 2.34	128	4.9	12.79	+ 0.82
264	280	18.1	25.18	82	15.1	22.87	— 2.31	42	20.1	26.64	+ 1.46
266	534	9.0	17.46	143	12.3	20.53	+ 3.07	146	9.3	17.76	+ 0.30
268	560	4.9	12.79	139	8.4	16.85	+ 4.06	143	3.4	10.63	— 2.16
272	578	13.6	21.64	111	10.8	19.19	— 2.45	135	14.9	22.71	+ 1.07
276	291	13.2	21.30	57	20.3	26.78	+ 5.48	119	9.9	18.34	— 2.96
277	573	2.7	9.46	149	2.8	9.63	+ 0.17	133	3.6	10.94	+ 1.48
Totals	5,418	100.6	187.59	1,304	116.3	199.56	+22.33	1,373	108.4	193.58	+13.02
							—11.36				— 5.67
Means	492.5	9.1	17.56	118.5	10.6	19.00		124.8	9.8	18.24	
Sums of Squares							146.0649				43.3539
						1963 test					
336	479	14.5	22.38	150	8.1	16.54	— 5.84	129	13.6	21.64	— 0.74
353	545	16.2	23.73	124	12.4	20.62	— 3.11	138	12.8	20.96	— 2.77
362	491	12.0	20.27	135	10.4	18.81	— 1.46	141	10.0	18.44	— 1.83
363	406	10.7	19.09	139	10.5	18.91	— 0.18	132	8.6	17.05	— 2.04
367	546	14.9	22.71	140	14.5	22.38	— 0.33	121	16.8	24.20	+ 1.49
Totals	2,467	68.3	108.18	688	55.9	97.26	—10.92	661	61.8	102.29	+ 1.49
											— 7.38
Means	493.4	13.7	21.72	137.6	11.2	19.55		132.2	12.4	20.62	
Sums of Squares							46.0506				17.9511

[1]) Percents adjusted to prevent discontinuities caused by small numbers of seedlings per plot (Cf. BARTLETT 1936 and 1947).

[2]) Percents transformed into angles equal to the arcsins of the $\sqrt{\%}$'s (Cf. BARTLETT 1947).

Table 5. — Analysis of variance in the standard crosses and in the 4- and 10-pollen mix crosses, 1962 test[1]).

Source of variation	Degrees of freedom	Mean square	F (vs. error)
	Standard cross		
Plus trees	10	808.43	**19.07
Testers	3	445.05	**10.50
Replicates (plots)	9	381.91	**9.01
Error	416	42.39	
Total	[2]) 483		
	4-pollen mix cross		
Plus trees	10	331.37	**4.37
Replicates (plots)	9	95.50	1.26
Error	89	75.76	
Total	[2]) 108		
	10-pollen mix cross		
Plus trees	10	271.79	**3.86
Replicates (plots)	9	133.76	1.90
Error	88	70.49	
Total	[3]) 107		

[1]) Basic plot data are percents of healthy seedlings, adjusted and transformed using the methods of BARTLETT (1936 and 1947).

[2]) Total excludes 1 missing plot of 1 cross, missing plot value estimated by method of SNEDECOR (1946, p. 268).

[3]) Total excludes 2 missing plots of 2 different crosses, estimated as above.

** Significant at the 1 percent level probability.

accurate mixed-pollen crosses, extremely high accuracy is even more unlikely. As a test of this reasoning, an analysis of variance was made on the 1962 test data *(Table 5)* to compare magnitude of error variance in the standard cross and the 4-pollen and 10-pollen crosses.

The lower "F's" for the mixed-pollen crosses showed that, proportionally, error variance is four to five times greater (4.37 and 3.86 against 19.07) in the mixed-pollen crosses. Here the effects of individual tester pollens could not be isolated, and the total number of plots and seedlings included in the test progenies was about one-fourth that of the standard crosses. In all three types of crosses, however, tests were sufficiently sensitive to distinguish significant effects of plus trees.

Thus, error variance was shown to be substantial, but probably not great enough to invalidate test results. What level of accuracy in selection is required by the practical tree breeder? Probably he would be quite willing to sacrifice some accuracy in return for the potentially large increases in test efficiency accruing to the mixed-pollen crosses. It must be recognized also that even if a tree breeder was using a low-accuracy method, he would achieve greater accuracy than might be expected, since he would be selecting at the upper limits of the percent-healthy distribution. Until he approached the selection cutoff point, he would probably be choosing most of the trees that were actually highest in combining ability.

For these reasons, acceptable levels of accuracy were set quite low (by experimental standards) — at ± 3 percent in the standard cross mean percent healthy values. Note *(Table 4*, column 3) that in the 1962 test, mean percent of healthy seedlings in progenies of the best three trees ranged between 13.2 and 18.1 percent. Thus the 3 percent accuracy actually amounts to almost 23 percent of 13.2 percent, and to almost 17 percent of 18.1 percent.

These criteria for reliability were then applied in the chi-square analyses advanced by FREESE (1960). By this method, accuracy of values given by a new technique ("observed" values) can be estimated by comparison with those "expected" on the basis of a presumably more accurate standard technique. In these experiments, expected values were the transformed percentages of healthy seedlings obtained using the standard cross, while observed values were corresponding percentages from the 4-pollen or 10-pollen crosses.

Once the desired level for accuracy (± 3 percent in 95 out of 100 cases) was set, the next step was to calculate the hypothesized variance for that level of accuracy, thus:

$$\frac{(\pm 3\%\text{, in arcsins, above and below standard cross average \% healthy})^2}{(1.96\text{, the standard normal deviate at the 5\% probability level})^2}$$

In the 11-tree test, then, where mean percent healthy for the 11 standard crosses was 9.1, the value of ± 3 percent in arcsins was computed as follows:

	Percent healthy	Arcsin	Difference	Average
Standard cross + 3%	12.1	20.36	2.80	3.03,
Standard cross %	9.1	17.56	3.26	
Standard cross −3%	6.1	14.30		

and hypothesized variance was computed as $3.03^2/1.96^2 = 2.3899$. Corresponding variance for the five-tree test (standard cross mean 13.7 percent) was 1.6531.

Before the analysis could be completed, however, it was necessary to consider the bias evident in the mixed-pollen results (columns 8 and 12, *Table 4*). For instance, in the 1962 test about two-thirds of the observed-expected differences were positive in sign, and the totals showed about twice the amount of plus deviations as of minus deviations. In the 1963 test the direction of the bias was reversed, there being an even heavier preponderance of minus values. Correlation analysis showed that the bias was not significantly associated with the magnitude of the expected, or standard cross values, but rather that it was more or less constant.

Fortunately, FREESE's paper considers this situation (pp. 141−142), giving a chi-square formula for making an approximate test of accuracy after elimination of bias. The formula is

$$\chi^2_{(n-1)\text{ d. f.}} = \frac{\text{Sum square obs.} - \text{exp. diffs.} - n(\text{mean exp.} - \text{mean obs. value})}{\text{Hypothesized variance}}$$

Thus for the 1962 (11-tree) test of the 4-pollen mix, using adjusted and transformed percents,

$$\chi^2_{10\text{ d. f.}} = \frac{146.0649 - 11(17.56 - 18.24)^2}{2.3899} = 51.5734,$$

and for the 1963 (5-tree) test of the 4-pollen mix, $\chi^2_{4\text{ d. f.}} = 9.4171$. Since the chi-square value for the 11-tree test exceeded the tabular chi-square at the appropriate degree of freedom and accuracy level (18.307), in this test the 4-pollen cross was judged incapable of meeting the accuracy criteria that had been imposed. In the 1963 5-tree test, however, chi-square was calculated as 9.4171, while the tabular value (9.488) was not exceeded. In this test, therefore, the 4-pollen cross was judged as barely capable of meeting imposed accuracy criteria.

Both of the 10-pollen mix crosses met prescribed accuracy levels. Here chi-square for the 1962 experiment was calculated as 16.0122; for the 1963 experiment it was calculated

Table 6. — Summary of heterogeneity chi-square analyses[1])

Year of experiment	Item	d. f.	4-pollen mix crosses		10-pollen mix crosses	
			χ^2	Probability of a greater χ^2	χ^2	Probability of a greater χ^2
1962	Total χ^2	11	20.334	< 5%	5.970	>80%
	Pooled χ^2	1	14.229		3.295	
	Heterogeneity χ^2	10	6.105	>70%	2.675	>98%
1963	Total χ^2	5	7.412	<20%	2.534	<80%
	Pooled χ^2	1	4.641		2.040	
	Heterogeneity χ^2	4	2.771	>50%	0.494	>95%

[1]) Using observed-expected differences in numbers of healthy and diseased seedlings with individual differences adjusted according to method of YATES (1934). Percents adjusted and transformed after the method of BARTLETT (1936 and 1947).

as 4.9797. Tabular values of chi-square (given above) were greater for both experiments.

The heterogeneity chi-square analyses of *Table 6* also led to the conclusion that the 10-pollen mix cross results were the more accurate. In the analysis of the 10-pollen crosses, the observed-expected differences fluctuated about as expected due to normal sampling deviations. They were quite homogeneous, there being only a 2 to 5 percent chance that as samples they fluctuated more widely than a normal chi-square distribution. In the analysis of the 4-pollen crosses, however, observed-expected differences were found to comprise a less homogeneous group of values. This fact was considered meaningful but not strong evidence (see SNEDECOR 1946, p. 192) that the 4-pollen cross results corresponded less closely to the standard crosses than did the 10-pollen cross results.

Practical considerations. — Experiments outlined here lead to the conclusion that in rust resistance breeding programs using large-scale tests of progeny, mixed-pollen cross progenies give reasonably reliable results. They are an economical substitute for the several individual crosses ordinarily used to determine general combining ability. Breeders using mixed-pollen crosses probably should include 10 or more pollens in the mixture. They should also realize that if mixed-pollen crosses are used, selection will be subject to error greater than that of tests using several individual crosses. Undoubtedly a tree breeder will select a few trees not quite the best, and cull a few trees which he might have selected using results of conventional tests. Also, he will be unable to select for specific combining ability.

Table 7. — Ranking and selection of plus trees under the 10-pollen cross and the standard cross.

Plus tree	10-pollen cross			Standard cross		
	Proportion healthy seedlings	Rank	Trees selected	Proportion healthy seedlings	Rank	Trees selected
	(Percent)			(Percent)		
	11-tree test					
61	15.9	2	×	10.5	5	×
69	14.9	4	×	11.8	4	×
70	7.1	7		7.6	7	
224	4.4	9		4.9	8	
257	4.9	8		4.3	10	
264	20.1	1	×	18.1	1	×
266	9.3	6		9.0	6	
268	3.4	11		4.9	9	
272	14.9	3	×	13.6	2	×
276	9.9	5	×	13.2	3	×
277	3.6	10		2.7	11	
	5-tree test					
336	13.6	2	×	14.5	3	
353	12.8	3		16.2	1	×
362	10.0	4		12.0	4	
363	8.6	5		10.7	5	
367	16.8	1	×	14.9	2	×

Pooled r_s (rank correlation) = 0.956, significant at the 1% level.

It was also of practical value to consider how we could increase accuracy in the mixed-pollen crosses without appreciably increasing their cost. Here, the analysis of variance *(Table 5)* was useful, in that the ratio of error variance for the mixed-pollen crosses to error variance for the standard crosses could be used to estimate the number of samples (plots) required in the mixed-pollen crosses to provide accuracy equal to that of the standard crosses (i. e., to equalize standard errors in the two types of crosses). Because

$$(\text{Standard error of the mean})^2 = \frac{\text{Error variance}}{\text{Number of samples}}$$

the equivalent number of plots (indirectly seedlings) required in the mixed-pollen crosses is

$$\sqrt{\frac{\text{Error variance of mixed-pollen crosses}}{\text{Error variance of standard crosses}}}.$$

For the 4-pollen mix crosses, the number of plots required was $\sqrt{75.76/42.39}$, or 1.34. Thus 1.34×10, or 13 plots were required, and roughly 1.34×118.5 (mean number of trees tested per 4-pollen mix cross, *Table 4)*, or 159 seedlings were required. For the 10-pollen mix crosses, where error variance was only slightly smaller, but where a few more trees were tested per cross, the required number of plots was $\sqrt{75.76/42.39}$, that is, 1.29×10, or 13 plots or 161 seedlings.

Thus, although there was little difference in the error variance of the 4-pollen and the 10-pollen crosses, it appeared that accuracy of either cross could be increased to standard cross levels by including three more plots. Since there were on the average 12.3 seedlings per plot in these tests, this 3-plot increase would amount to about 40 more

seedlings per progeny. This improvement, along with prevention of patchy inoculation, would go far toward increasing overall reliability of mixed-pollen crosses.

Summary

Results and analyses given in this paper show that mixed-pollen crosses which involve 10 or more pollens can be used to obtain relatively reliable estimates of general combining ability and breeding value of blister rust resistant plus trees. Mixed-pollen cross results fell within ± 3 percent of those obtained with more conventional tests using four individual tester crosses, in 95 out of 100 cases. This level of accuracy is low by experimental standards, but it is perfectly adequate for large-scale, practical plus tree testing. Prevention of patchy inoculation of progeny test plots, as well as an increase in numbers of plots tested in mixed-pollen cross progenies, would further improve accuracy.

Literature Cited

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A Comparative Karyotype Analysis of Pseudotsuga menziesii (Mirb.) Franco, and Pseudotsuga wilsoniana (Hayata)

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(Received for publication June 27, 1967)

On a morphological scale, the karyotype of a species can be characterized according to number, total length of the individual chromosomes comprising the set, and position of primary and secondary constrictions. The karyotype of a species is mostly a fixed character, but over a long time karyotypes undergo gradual evolution. Moreover, different species within a genus frequently have visibly different karyotypes; comparisons of chromosome morphology between related species can yield valuable information on the processes of evolution and serve as valuable tools to the taxonomist (SWANSON 1964).

Six species are currently recognized in the genus *Pseudotsuga* (DALLIMORE and JACKSON 1948, GÖHRE 1958, LI 1953). These are Douglas-fir *(P. menziesii* [MIRB.] FRANCO), bigcone Douglas-fir *(P. macrocarpa* MAYR), Japanese Douglas-fir *(P. japonica* BEISSNER), Formosan Douglas-fir *(P. wilsoniana* HAYATA), Chinese Douglas-fir *(P. sinensis* DODE), and Forest's Douglas-fir *(P. forrestii* CRAIB). Among these species, karyotypes have been reported on only two of them, *P. menziesii* and *P. macrocarpa*. The present study is of the karyotypes of *P. wilsoniana* and *P. menziesii* and presents a comparative karyotype analysis for all three species.

Review of Previous Work

The first major publication on chromosome morphology among the conifers was by KARL and HALLY SAX in 1933. They found a basic number of n = 12 for most of the specimens they studied, with two exceptions in the *Pinaceae, Pseudolarix,* with n = 22, and *Pseudotsuga,* with n = 13. They attributed the extra chromosome in *Pseudotsuga,* which was apparently telocentric, to segmental interchange and duplication. Besides the telocentric chromosome, they found six metacentric chromosomes and six submetacentric chromosomes.

ZENKE (1953) studied meiosis in *P. menziesii* var. *viridis (= menziesii).* She reported 13 bivalents at diakinesis of meiosis, which confirmed the findings of SAX and SAX (1933). Five pairs of bivalents were large with apparently median to submedian centromeres; six pairs were smaller and apparently had subterminal centromeres. The exact classification of the two smallest pairs could not be accurately discerned.

ANDREE DURRIEU-VABRE (1958) found n = 12 chromosomes in root tip metaphases of *Pseudotsuga douglasii (= menziesii).* She expressed each chromosome in terms of a ratio (R) in which the numerator was the value of the shorter arm and the denominator that of the longer arm. Her results showed that ratios of three chromosome pairs were 1 : 1; of two pairs, 3 : 4; of one pair, 2 : 3; of four pairs, 1 : 2; and of two pairs, 1 : 3.

BARNER and CHRISTIANSEN (1962) published findings on the chromosomes of *P. menziesii* var. *viridis.* They reported a haploid number 13, which agreed with the findings of SAX and SAX (1933) and ZENKE (1953). The 13 chromosomes consisted of five approximately isobrachial chromosomes, six heterobrachial chromosomes, and two apparently telocentric chromosomes. Their examinations were made on both meiotic and mitotic figures, and, at times, they observed meiotic irregularities of pairing and of anaphase separation. These irregularities did not occur with great frequency, and most cells were normal.

CHRISTIANSEN (1963) studied root-tip mitoses of *P. macrocarpa* and reported 2n = 24 for that species. The karyotype included six pairs of metacentric chromosomes and six pairs with subterminal centromeres.

Materials and Methods

Two seed lots, one of Douglas-fir and one of Formosan Douglas-fir, were used for all investigations made. Both seed lots were from single-tree collections. Seeds of Douglas-fir came from a tree near Corvallis, Oregon; seeds of

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