namo 16, Anhang 1–6, 1941. — **Schulz**, A.: Beiträge zur Morphologie und Biologie der Pflanzen. Ber. d. D. bot. Ges. 10, 303–313 (1892). — **Stern**, K.: Rassenbildung und Bestandesanerkennung. Z. Forstgenetik 5, 126 (1956). — **Stevens**, F. L.: Teratology. Bull. Torrey Bot. Cl. XXI, 490 (1894). (cit. nach Penzig.) — **Ustinowa**, E. I.: Einige Besonderheiten der Bildung generativer Organe in den Blütenständen des Maises. Botan. Journ. 41, 864–867 (1956). *[Russisch.]*

Selective Fertilization in Pinus monticola Dougl.

I. Preliminary Results

By A. E. Squillace and R. T. Bingham[1])

(Received for publication February 15, 1958)

In a previous report (Bingham and Squillace, 1955) the authors showed that self-fertility (ability to produce germinable self-fertilized seed) in western white pine (Pinus monticola Dougl.), as tested under controlled pollination, varied greatly among individual trees and occasionally was rather high. Self-pollinated seedling yields averaged about 50 percent of cross-pollinated yields, but in 4 of 28 trees tested they almost equalled or exceeded cross-pollinated yields. It was also shown that selfed seedlings exhibited an appreciable degree of inbreeding depression. The results agreed with findings of workers with many other forest tree species.

Knowledge of potential self-fertility and resultant inbreeding depression has caused concern among forest tree improvement workers, especially since the end-product of many tree improvement ventures is the establishment of seed orchards with members of superior clones (Jensen, 1945; Langner, 1953; Langner and Stern, 1955). It has been reasoned that in such orchards more selfing could occur than in natural stands. Not only could each member be selfed by itself, but also by all other members of the same clone. However, there is also the possibility that although many trees are self-fertile as tested under controlled self-pollination, self pollen might be discriminated against when in competition with other pollens as under natural pollination conditions. Such discrimination, if found to occur, may largely eliminate selfing among members of the same clone in seed orchards.

These recent findings on self-fertility and their implications prompted a study of selective fertilization in western white pine. Preliminary phases of this study have been completed and are reported upon in this paper.

Jones (1928) used the term "selective fertilization" to cover broadly all types of discrimination in reproduction. In this sense, it includes any selective action occurring in any stage of the reproductive cycle. The discrimination may occur among different gametes of the same individual, among gametes of different individuals of a given species, or among gametes of individuals of different species. Thus, incompatibility (inability to effect fertilization) often occurring between individuals of different species may be considered as selective fertilization in its broadest sense. Within species, differences in time of flowering, rate of pollen tube growth, and various other factors could result in selective fertilization. In a more strict sense, however, the term implies an inequality of fertilizing power of gametes of differing genetic constitution.

The present study is concerned with the relative ability of a self-fertile tree to produce germinable selfed seed when its pollen must compete with that of another tree. Differences in the proportion of germinable "selfed" vs. "crossed" seeds produced under these conditions may be due to differences in the relative ability of the two pollens to germinate, to extend their tubes, to fertilize the egg, or to any combination of these factors. The study, designed mainly to evaluate the over-all efficacy of the two competing pollens, permits only speculation in regard to which of the factors were operative.

Review of Literature

Limited review of the literature on selective fertilization in agricultural plants showed that considerable research had been done in this and related fields. The phenomenon has been shown to occur in various forms in many species. Discrimination of female flowers of a given plant or line for or against its own pollen when in competition with pollen from another plant or line has received special attention. For example, Jones (1928) showed that maize plants (highly self-fertile within lines) of a given line usually discriminated against pollen of other lines. On the other hand, East (1919) found that *Nicotiana* plants of a given strain (highly self-sterile) completely favored pollen from other strains. In many plants differences in rate of pollen tube growth were found to account for discrimination, and often other factors such as abortion of the gametes or zygotes were involved.

Comparatively little work has been done on selective fertilization in forest trees. Competition occurring among immature embryos within the same ovules in various conifers was discussed by Buchholz (1926), and Stockwell (1939) pointed out the various means by which natural selection might occur prior to embryo maturation in pines. Piatnitsky (1948) found that in oaks (*Quercus* spp.) pollen from other trees germinated "better" on stigmas of a given tree than did self pollen and concluded that cross-pollination is favored to some extent over self-pollination.

Philp and Sherry (1946) showed that although green wattle (Acacia decurrens Willd.) is self-fertile, it was naturally cross-pollinated to the minimum extent of between 85 and 95 percent under open-pollination in plantations.[2]) Their technique for determining the degree of natural crossing is interesting. Certain parent trees were shown to produce self-pollinated progenies that segregated for several recessive leaf characters. Open-pollinated progenies also segregated for the same characters but in dif-

---

[1]) A. E. Squillace, Forester, Inland Empire Research Center, Intermountain Forest and Range Experiment Station, U. S. Forest Service, 157 South Howard Street, Spokane 4, Washington. — R. T. Bingham, Forester, Spokane Unit, Blister Rust Control, U. S. Forest Service, Region I, 157 South Howard Street, Spokane 4, Washington.

[2]) In regard to the extent of self-fertility, 27 percent of ovules set seeds under controlled self-pollination compared to 72 percent under open-pollination, and germination was 9 percent less among selfed seeds.

ferent ratios. The divergence of segregating ratios in open-pollinated progenies from corresponding ratios in self-pollinated progenies permitted an estimate of the degree of natural crossing. After using a similar technique, MOFFETT (1956) showed that minimum natural crossing in black wattle (*A. mollissima* WILLD.) ranged from 62 to 97 percent, with a mean of 84 percent.

Experimental Procedure

The basic technique used in the present study was patterned somewhat after the method used by JONES (1920 and 1928). His technique involved applying a mixture of pollen from two plants (or lines) A and B to female flowers of both A and B. Possible progenies are A×A, A×B, B×A, and B×B. Hypothetically, the ratio of seeds A×A to A×B should be equal to the ratio of seeds B×A to B×B, irrespective of any differences in numbers of functional pollen grains in the mixture. If the ratios are not equal, a selective action favoring either selfing or crossing is indicated. As JONES pointed out, however, the method merely measures the combined excess of selfing over crossing or vice versa. It can be used effectively when the selective fertilization in both plants occurs in the same direction, either in favor of selfing or crossing. However, if plant A actually favors selfing and plant B favors crossing, the net result of equal but opposite effects could produce roughly similar character ratios indicating a lack of selective fertilization, when it had in fact occurred — in opposite directions. The procedure was satisfactory in JONES' maize experiments where a large number of trials showed an average discrimination in one direction. If there is lack of preliminary information regarding the direction of discrimination and the investigator is interested in the performance of individual plants, a modification of technique is required. The modified procedure used in the present tests is discussed here. Further modifications for improving future tests will be discussed later in this paper.

In designing the present tests the problem of determining the proportion of selfed vs. crossed individuals in a progeny resulting from a mixed pollination had to be solved. No specific foliar or other readily recognized segregates were known in western white pine. However, as already discussed, breeding among a number of western white pine selections[3]) showed that certain trees, when selfed, produced progenies that were considerably stunted, especially when they were compared to the more vigorous progenies from outcrosses of the same trees. For example, previous testing showed that selection 58×selection 18 (selection number 58 used as the female parent in a mating with number 18 as the pollen parent) yielded one-year-old seedlings averaging 36 percent taller than those from 58×58. Therefore, it was believed that if the self and cross were made along with the mixed pollination, their differential growth could be used as a means of determining the proportion of selfs versus crosses in the mixed progeny. Admittedly, by this method it is not possible to determine whether each individual seedling resulting from the mixed pollination is a cross or a self. However, it was believed that with careful appraisal of the effect of seed weight and germination time upon growth, at least a good estimate of the proportion of selfs vs. crosses occurring in the mixed progeny could be made by comparing the mean growth rates of the mixed progeny with those of the corresponding self and cross progenies. It was further believed that other factors such as seed yield and cotyledon number might be employed in determining the nature and in estimating the degree of selective fertilization that occurred. Details of the procedure follow.

In the spring of 1954 controlled pollinations were made among 3 western white pine trees, selection numbers 18, 19, and 58, as shown below. The matings were grouped into 3 parts considered as separate tests.

Test 1: 19×19, 19×58, and 19×(19+58),
Test 2: 58×58, 58×18, and 58×(58+18),
Test 3: 58×58, 58×19, and 58×(58+19).

Note that in each test an individual tree is selfed, crossed with another tree, and finally pollinated with a mix containing self pollen and pollen from the tree with which crossed. The pollen mix in each test was an approximate 1:1 mixture by volume. The self-pollination 58×58 shown for both tests 2 and 3 was not actually done twice; rather, the data obtained from the single pollination were used in analyzing results for both tests. The objective of each test was to estimate the relative proportion of selfs and crosses resulting from use of the mixed pollen in each case.

The controlled pollinations were fairly successful and cones were collected in the fall of 1955. Seeds were extracted and winnowed, with counts made for both full and blind seeds. Lengths of cones were measured. Full seeds were then weighed individually with sufficient accuracy to place them into 3-milligram weight classes. These seed lots, comprising different matings and different seed weights within each were kept intact throughout the remainder of the experiment.

Seeds were sown in randomized blocks in a nursery at Coeur d'Alene, Idaho in May 1956. Germination and mortality checks were made at 24, 38, 53, and 107 days after sowing. The number of cotyledons on all seedlings emerging within 53 days after sowing was counted. Finally the length of the epicotyl (portion of stem above cotyledons) and total height were measured on all surviving seedlings in September 1956. It was felt that epicotyl length would reflect inherent differences among progenies better than total height; hence it was measured along with total height.

Among progenies having a common seed parent, mean total seed yield (sound plus blind seeds) per cone was found to be significantly and positively correlated with mean cone length. Therefore, it was necessary to compute adjusted total yield per cone by using mean cone length (171 mm) and the pooled regression (b = .367) of seed yield on cone length within seed parents. Finally, adjusted yields of sound and blind seed per cone were computed from adjusted total yield per cone on the basis of percent soundness (*table 1*). Thus, the sound and blind seed yields per cone shown in table 1 are adjusted values, presumably representing those that would have been attained had all cones been equal in length.

Seedlings emerging in the seed beds after the 24th day following sowing possessed shorter epicotyls on the average and were shorter in average total height than those emerging earlier. Since the percentage of such seedlings was relatively small (*table 1*) they were omitted in analyses involving height growth. It was realized that total emergence was not complete: some seeds probably had germinated but had not emerged to the point of being visible above ground, while other seeds might be capable of germinating in the future. The effect of these factors

---

[3]) Trees selected for resistance to blister rust, *Cronartium ribicola* FISCHER, are discussed in a report by BINGHAM et al. (1953.)

Table 1. — Cone and seed yield, seed soundness and weight, and seedling emergence and mortality, by matings

Mating	Flowers pollinated	Cones matured 1)	Total sound seed yield	Adjusted sound seeds per cone	Adjusted blind seeds per cone	Seed soundness 2)	Mean sound seed weight	Seedling emergence		
								Within 24 days 3)	After 24 days 4)	Mortality 5)
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*	*Milligrams*	*Percent*	*Percent*	*Percent*
					Test 1					
19 × 19	26	19	588	26.9	55.1	32.8	22.4	53.5	4.8	17.0
19 × 58	23	20	1,267	59.9	12.6	82.6	21.2	60.6	3.4	10.3
19 × (19 + 58)	26	21	1,268	56.0	21.2	72.6	21.8	46.0	4.4	15.1
					Test 2					
58 × 58	16	14	1,187	88.0	46.2	65.6	18.1	51.1	1.2	21.1
58 × 18	12	4	366	90.6	18.3	83.2	20.2	33.0	2.8	14.9
58 × (58 + 18)	12	4	421	113.4	8.9	92.7	14.9	54.3	2.3	13.1
					Test 3					
58 × 58	16	14	1,187	88.0	46,2	65.6	18.1	51.1	1.2	21.1
58 × 19	11	8	454	63.8	50.5	55.8	14.6	45.8	2.5	17.7
58 × (58 + 19)	12	8	459	67.2	49.7	57.5	12.5	61.5	1.8	15.1

1) Losses indicated were mostly the result of insect damage — thus differences in the proportion of cones maturing in various matings were probably not pollen parent effects.

2) Sound seed expressed as a percentage of total (sound plus blind) seed.

3) Percent of seedlings emerging within 24 days after sowing.

4) Percent of seedlings emerging after 24th day following sowing.

5) First season mortality among seedlings which emerged within 24 days after sowing expressed as a percent of the total emerging within 24 days.

probably was small, but how they may have affected the results is not known. It is likely that they would tend to result in an underestimate of the degree of selfing because selfed seeds are known to exhibit a greater degree of defective germination and pre-emergence mortality (Bingham and Squillace, 1955). However, such effects upon the results probably are not very important from a practical standpoint because in seed orchards we would be concerned only with the proportion of functional selfed and crossed seedlings.

The lengths of epicotyls, total heights, and numbers of cotyledons for seedlings within a progeny usually were found to be correlated significantly with seed weight (*table 2*). Therefore, covariance analyses with adjustment for seed weight were used in testing significance of differences in mean length of epicotyl, total height, and number of cotyledons for progenies of various matings. In doing so, combined tests of significance were made for each of the 3 selective fertilization tests prior to testing individual pairs of matings.

Results

Test 1

Data on practically all factors studied strongly suggest that the use of pollen mix from trees 19 and 58 on female fowers of tree 19 resulted in production of more cross (19 ×58) than self (19×19) seed and seedlings. Original data on epicotyl length are plotted over seed weight in *figure 1*. Note that for each progeny epicotyl length increased with seed weight, and that the plotted line representing the two factors for the mixed progeny falls closer to the cross than to the self. Covariance analyses and adjustments were made on these data to eliminate seed weight effects statistically. (Regression and correlation coefficients are shown

Table 2. — Regression (b) and correlation (r) coefficients of length of epicotyl, total height, and number of cotyledons on seed weight for progenies of each mating1)

Mating	Length of epicotyl		Total height		Cotyledon number	
	b	r	b	r	b	r
			Test 1			
19 × 19	0.298**	0.25**	0.425**	0.29**	0.068**	0.21**
19 × 58	.311**	.19**	.983**	.51**	.037**	.15**
19 × (19 + 58)	.198**	.15**	.412**	.26**	.013	.07
Pooled2)	.254**	.19	.628**	.37**	.029**	.12**
			Test 2			
58 × 58	.561**	.21**	.858**	.26**	.067**	.18**
58 × 18	1.061**	.30**	.881*	.22*	.062	.14
58 × (58 + 18)	1.350**	.35**	1.416**	.35**	.116**	.27**
			Test 3			
58 × 58	.561**	.21**	.858**	.26**	.067**	.18**
58 × 19	.312	.14	1.029**	.36**	.138**	.38**
58 × (58 + 19)	.199	.08	.055	.02	.097**	.25**
Pooled3)	.617**	.22	.826**	.24**	.088**	.23**

1) Units of measure for length of epicotyl and total height are millimeters, and for seed weight, milligrams.

2) Pooled within-groups regression and correlation coefficients for all matings made on seed tree 19.

3) Pooled within-groups regression and correlation coefficients for all matings made on seed tree 58.

** Significant at the 1 percent level.

* Significant at the 5 percent level.

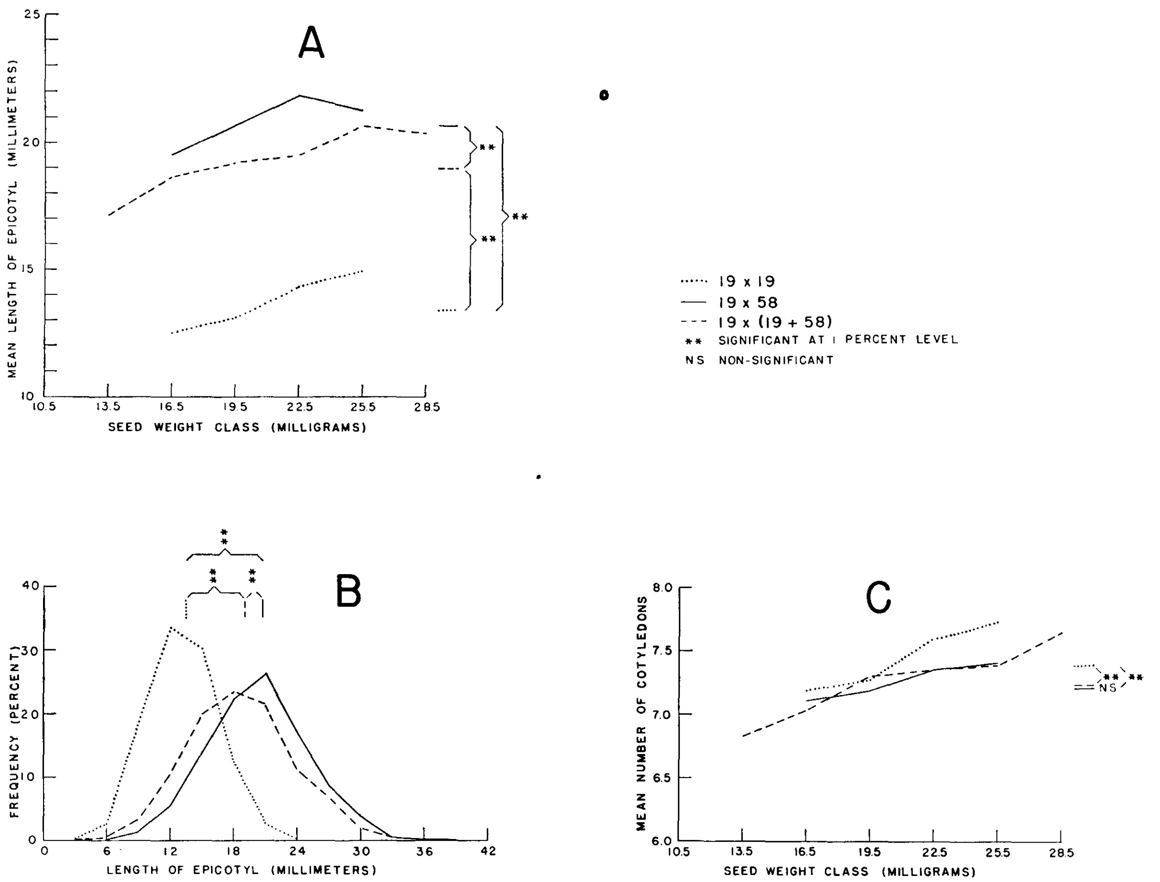

Figure 1. — Results of test 1. — A, Epicotyl length vs. seed weight. — B, Frequency polygon of adjusted epicotyl length. — C, Cotyledon number vs. seed weight. — Progeny means, after covariance adjustment, and significance of their differences are shown on the right or upper side of each figure.

in *table 2.)* The adjusted mean epicotyl lengths, representing values that presumably would have been obtained had all seeds been of equal weight (seed weight class 19.5 mg.), are shown in *table 3.* Note that mean epicotyl length for the self progeny was 13 millimeters, the cross 21 millimeters, and the "mix" 19 millimeters. The difference between any two of these was highly significant. Note again that progenies resulting from the pollen mix were closer to the cross than to the self progenies in respect to mean epicotyl length. One might estimate that roughly one-fourth (21—19/21—13 = .25) of the seedlings from the mixed mating were selfs and three-fourths crosses.

A frequency polygon of adjusted epicotyl length is also shown in *figure 1.* It shows pictorially the reliability of both indicating an excess of crosses over selfs in the mix progeny. Large numbers of seedlings represented in each progeny of test 1 resulted in rather smooth distributions. Note that the self distribution tends to be peaked while

Table 3. — Adjusted[1]) mean length of epicotyl, total height, and number of cotyledons of one-year-old seedlings produced from each mating

Mating	Length of epicotyl			Total height[2])		Number of cotyledons		
	Adjusted mean	Signif. of differences	Basis, seedlings	Adjusted mean	Signif. of differences	Adjusted mean	Signif. of differences	Basis, seedlings
	Millimeters		*Number*	*Millimeters*		*Number*		*Number*
				Test 1				
19 × 19	13	**	249	31	**	7.39	**	314
19 × 58	21	**	664	39	**	7.22	NS **	759
19 × (19 + 58)	19	**	490	37	**	7.24		617
				Test 2				
58 × 58	22	**	463	38	**	7.80	NS	565
58 × 18	25	**	97	43	**	7.82	NS NS	120
58 × (58 + 18)	32	**	186	50	**	7.79		217
				Test 3				
58 × 58	22	NS	463	38	**	7.80	**	565
58 × 19	21	NS NS	163	40	NS NS	7.56	NS	191
58 × (58 + 19)	22		231	39		7.74	*	275

[1]) Adjusted for seed weight as discussed in text.

[2]) Basis (number of seedlings) same as for length of epicotyl.

** Significant at the 1 percent level.

* Significant at the 5 percent level.

NS Nonsignificant.

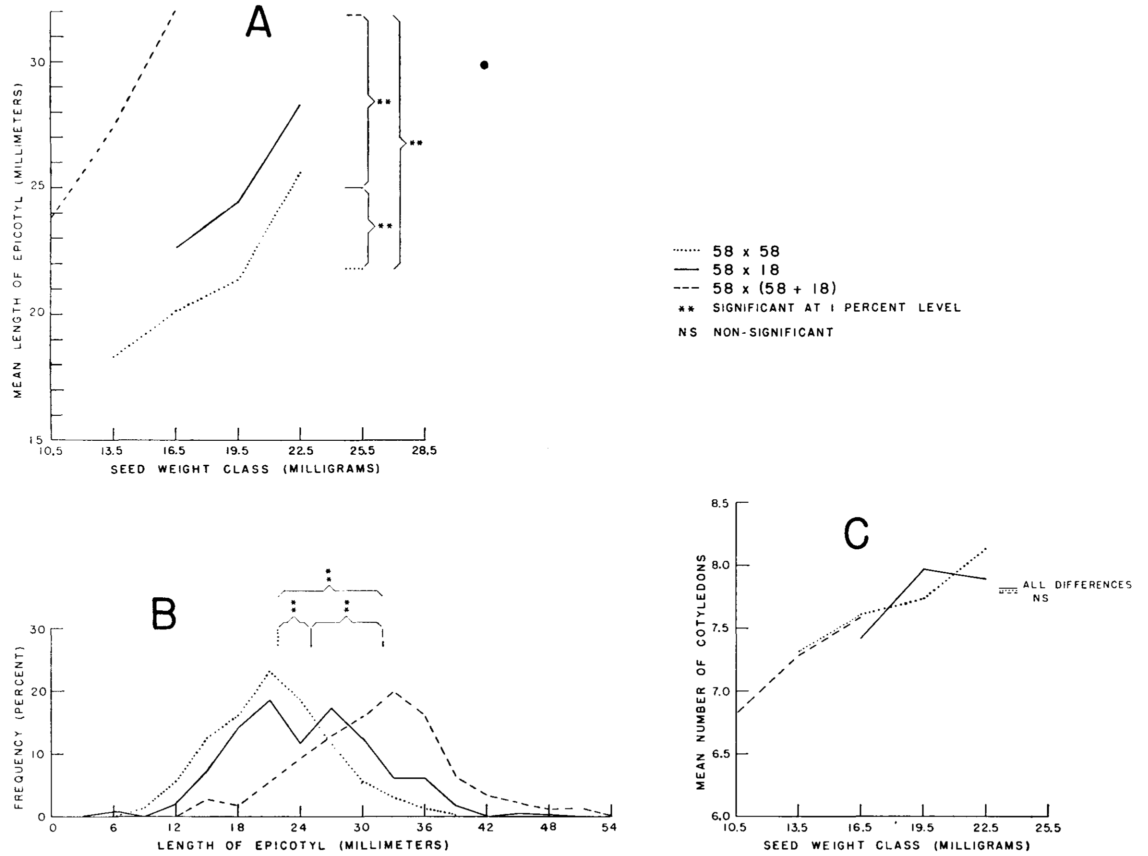

Figure 2. — Results of test 2. — A, Epicotyl length vs. seed weight. — B, Frequency polygon of adjusted epicotyl length. — C, Cotyledon number vs. seed weight. — Progeny means, after covariance adjustment, and significance of their differences are shown on the right or upper side of each figure.

both the cross and mix distributions tend to be more nearly normal or relatively flat-topped.

Data on adjusted average total height and cotyledon number yielded results similar to that for epicotyl length, both indicating an excess of crosses over selfs in the mix progeny *(table 3)*. Although differences in cotyledon number were very small they were significant. The realness of the differences in cotyledon number are more apparent in *figure 1*, where unadjusted mean cotyledon number is plotted over seed weight. Using total height, the proportion of selfs to crosses in the mix progeny was estimated to be 25:75; using cotyledon number, 12:88. These ratios are equal to or not greatly different from the 25:75 ratio obtained from use of epicotyl length, and the average of all three ratios is 21:79.

Note that sound and blind seed yield per cone and seed soundness for the mix progeny are closer to the cross than to the self progeny in each case. These results support the conclusion that crossing was favored over selfing in test 1.

Test 2

Data from test 2 were unusual in that progenies of the mating 58×(58+18) grew considerably more rapidly than either those of 58×18 or 58×58 *(figure 2* and *table 3)*. It is possible that the use of the pollen mix may have resulted in a selective action among individual gametes having factors for rapid growth rate in pollen No. 18 or in No. 58, or both. On the other hand, it is possible that progeny 58×18 grew slowly because of environmental factors. Matings 58×18 and 58×58 had been made in connection with other experiments in 1952, and in those tests progeny 58×18 grew an average of 36 percent taller than 58×58 in the first year. In the present test the cross grew only 13 percent taller than the self.

Irrespective of the apparent anomaly both mean epicotyl length and mean total height for the mix are nearer to the respective means for the cross than to those for the self *(figure 2* and *table 3)*. Thus it was concluded, with some reservation, that use of the mixed pollen resulted in more crosses than selfs, as in test 1. Seed yield and soundness data *(table 1)* are mostly in harmony with this conclusion. Note that in blind seed yield and seed soundness, where the cross and self are appreciably different, values for the mix are nearer to respective values for the cross than to those for the self. Test 2 probably should be repeated in its entirety.

Test 3

In test 3 differences in both epicotyl length and total height between 58×19 and 58×58 were so small *(figure 3* and *table 3)* that it was impossible to draw conclusions regarding the mix, 58×(58+19). Cotyledon number, on the other hand, presents fair evidence of an excess of selfing, the cross and the self being highly significantly different and the mix being significantly closer to the self than to the cross. Differences in sound and blind seed yield per cone and seed soundness were not great enough either to support or refute results based upon cotyledon number. Therefore it can be concluded with a fair degree of confidence that selfing was favored over crossing in test 3. Using cotyledon number, the ratio of selfing to crossing was estimated to be 75:25.

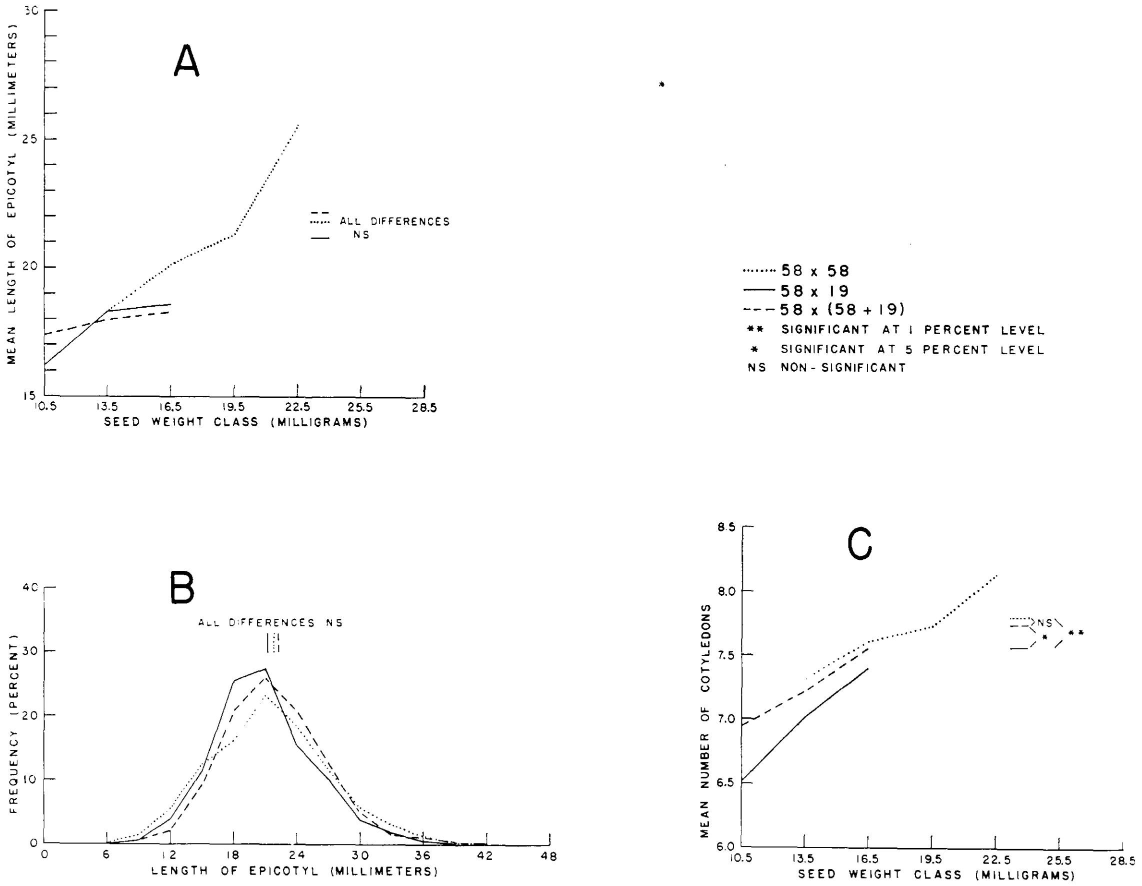

Figure 3. — Results of test 3. — A, Epicotyl length vs. seed weight. — B, Frequency polygon of adjusted epicotyl length. — C, Cotyledon number vs. seed weight. — Progeny means, after covariance adjustment, and significance of their differences are shown on the right or upper side of each figure.

Discussion

Evidence shown above indicates that in three tests involving different pairs of pollen parents crossing exceeded selfing in two of them, but the reverse was true in the third. A close look at the over-all results permits some speculation on the causes of the inequality of selfing and crossing occurring in the mix matings.

In test 1 the ratio of seed yield per cone for 19×19 vs. 19×58 was 31:69. The relatively low yield from 19×19 was not due to pollen viability or vitality because use of the same lot of pollen (19) on tree 58 yielded a much more nearly normal number of seeds per cone. Rather, it indicates a relatively low degree of self-compatibility for tree 19. Furthermore, in the selfed progeny, seedling emergence within 24 days was less (about 7 percent) and mortality greater (about 7 percent) than in the cross 19×58. Thus, even without competition in pollen tube growth in the mixed mating, 19×(19+58), the ratio of selfs to crosses hypothetically would be about 24:76. This is close to the actual average 21:79 ratio of self vs. cross seedlings determined from results of the test. Therefore, one might speculate that most of the excessive crossing occurring in the mixed mating of test 1 was not due to competition in growth of pollen tubes. Rather, it could have been due largely to the inability of pollen 19 to effect fertilization or to later misfunctioning or mortality of the zygotes after fertilization, in the same manner as when tree 19 is artificially selfed without competition with other pollens.

In test 2, the seed tree involved, 58, was highly self-compatible as indicated by the large number of seeds per cone produced by the self-pollination 58×58 in this and previous tests. Recall that the data indicated an excess of crossing over selfing, as in test 1. However, in this instance the excessive crossing can be ascribed neither to failure soon after fertilization nor to failure in germination. Mortality was about 6 percent greater in the selfed progeny, but this difference was not nearly enough to account for the apparent deficiency of selfs in the mix progeny. Similarly, the deficiency of selfs was not likely due to fertilization failure or zygote mortality because in the self mating, 58×58, where no competition was provided, seed and seedling yield was normal. One logical explanation was that actual competition between pollen grains of 18 and 58 occurred, in the nature of differential growth rate of pollen tubes or in some other manner, with pollen of tree 18 being favored.

In test 3 the highly self-compatible tree 58 was again used as the seed parent. On the basis of seed yield, seedling emergence, and mortality, one would expect a hypothetical ratio of about 59:41 in favor of selfing from use of the pollen mix. However, the tests gave a ratio of 75:25 in favor of selfing. Therefore, it might be concluded here that pollen competition was somewhat in favor of tree 58 and that some of the excessive selfing was accounted for by the high self-compatibility of tree 58.

The results suggest that the outcome of selective fertilization tests as conducted here depends largely upon the relative self- and cross-fertility of the particular trees involved. It is likely that self-sterile or moderately self-fertile trees usually favor crossing while highly self-fertile trees may favor either selfing or crossing depending upon cross-fertility and competition in pollen tube growth or other factors between the particular trees involved. If so, it might be hypothesized that western white pine trees under natural pollination conditions are largely cross-pollinated because: — (1) Most trees are less self-fertile than they are cross-fertile.[4]) — (2) Even in the relatively few highly self-fertile trees, crossing often may be favored over selfing because under natural conditions the wind carries a mixture of pollen from a relatively large number of trees, and it is probable that one or more of the pollens thus carried is more competitive than self pollen.

Height growth data on progenies produced by the authors in the years 1952 through 1955 for other experiments lend further support to the belief that in western white pine crossing is favored over selfing under natural pollination conditions. Among these progenies were included 20 selfs, the corresponding wind-pollinations, and a number of various crosses involving the same 20 trees. By comparing the growth rate of progenies produced through wind-pollination against those resulting from controlled selfing and crossing, one can speculate on the degree of selfing that occurs under natural, wind-pollination conditions. This was done, and it was revealed that growth rates up to 4 years of age among the wind-pollinated progenies were on the average just as great as the cross-pollinated progenies from the same seed parents, and that both wind- and cross-pollinated progenies grew an average of 22 percent faster than selfs. In 17 out of 20 cases growth rate of the wind-pollinated progenies exceeded that of the mean between the respective self and cross progenies.

These results should not lead to complacency in regard to the possibility of excessive production of selfed seedlings from seed produced on clone members in seed orchards. As mentioned earlier the potentiality for selfing in seed orchards is magnified. Further experiments are needed not only to test the hypothesis suggested by the present study but also to determine the degree of selfing that might occur under seed orchard conditions where the potentiality for selfing is greater than that in natural stands. It should be borne in mind that, regardless of the outcome of these experiments, certainly all ordinary methods of minimizing the degree of selfing, such as segregating individual clone members, should be employed to the extent possible. The experiments will have great value, however, in determining the need for employing special measures. For example, a very high degree of preference for selfing of certain individuals may necessitate their omission in the orchards. A general high degree of selfing may necessitate such measures as closer spacing to assure more cross-pollination, increasing the number of different clones, or even chemical or other economical forms of emasculation.

In designing future experiments several points should be borne in mind. First, there may be little need for testing selective fertilization in trees known to be relatively self-sterile under controlled pollination, at least from a practical standpoint. Repeated tests of self-fertility of clones considered for use in seed orchards will save a great deal of work and probably should have high priority.

A second consideration is that when phenological barriers to selfing are lacking, either in natural stands or in seed orchards, self pollen is likely to be more accessible to female flowers in a given tree than is pollen from other trees. The proportion of self vs. cross pollen reaching female flowers probably varies with tree spacing. At any rate, we need to know how a given excess of self pollen affects the degree of selfing in self-fertile trees. Similarly, how will the use of pollen mixtures containing pollen from a number of trees (say equivalent to the number of clones expected to be used in an orchard) affect the degree of selfing?

A third consideration involves the mating scheme to be employed. Unless foreknowledge of the segregation for certain characters is available, a mixed mating, such as A×(A+B), must be accompanied by the self (A×A) and cross (A×B) matings as done in the tests described. The reciprocal mating, B×(A+B), is not necessary. However, in order to account for differences in viability (germinative power) and vitality (ability to grow) of the pollens, the pollens used, say A and B, should be outcrossed onto at least one other tree, C (as done by Jones, 1920, in some of his selective fertilization tests). The proportion of seeds C×A to C×B would reflect to some degree differences in viability and vitality of pollens A and B. However, it may also reflect differences in cross-fertility of the matings C×A and C×B. To overcome this difficulty critical tests will require outcrossing onto more than one tree and averaging of the results. At any rate the proportion of selfs to crosses resulting from the mixed mating could be adjusted to account for differences in pollen viability and vitality determined by outcrossing.

The reader may question why pollen germination tests in vitro commonly conducted are not considered sufficient for use here. Admittedly, such tests should be made because they may be useful in explaining end results, such as when a previously tested, compatible mating fails on a repeated attempt. However, these tests alone may often be insufficient because they do not measure pollen vitality. Pollen grains may germinate under a laboratory test, but because of storage or other environmental effects they may not possess their normal ability to grow. For example, Orr-Ewing (1956) found that although fresh and one-year-old pollen germinated equally well in laboratory tests, the latter resulted in drastically reduced seed yield compared to yield resulting from the same lot of pollen used the year before on the same seed tree. Although the differences in yield could have been due to a seasonal effect on normal seed production, it could well have been due to reduced pollen vitality. Thus it is felt that critical selective fertilization experiments should employ outcrossing as suggested above for accounting for differences in viability and vitality of pollen, in addition to germination tests in vitro.

With the above considerations in mind the following procedure is tentatively planned for use in estimating the degree of selfing that might occur in a seed orchard comprised of several clones. To test, for example, the degree of selfing by self-fertile tree A when its own pollen is in competition with a number of other pollens ("multiple mix"), say equivalent to the number of clones expected in seed orchards, the following mating scheme is planned: A×A, A×multiple mix, and A×(A+multiple mix). Then, to test pollen viability and vitality, the pollens "A" and "multiple mix" will have to be crossed with several other trees (other than A or those used in the "multiple mix" pollen). The "multiple mix" pollen should, of course, be comprised of equal parts of fresh pollen from all trees represented, collected and extracted under the best possible conditions. In the mating A×(A+multiple mix) how-

---

[4]) As mentioned earlier, Bingham and Squillace (1955) found that selfing resulted in an average of 50 percent fewer germinable seeds than did crossing. Higher seedling mortality following germination among selfs as found in the present tests would increas the difference further.

ever, the quantity of A pollen must be greater than that represented by each of the pollens constituting the "multiple mix" in order to allow for the greater accessibility of self vs. cross pollen as discussed earlier. The magnitude of this allowance probably would have to be estimated on the basis of pollen dispersion studies.

Need for careful experimental control in selective fertilization tests cannot be overstressed. For example, note the great differences in average seed weight among different pollinations made on the same seed tree, such as 18.1 mg. for 58×58 and 12.5 mg. for 58×(58+19) *(table 1)*. Such differences necessitated accurate determinations of the effects of seed weight, including weighing of each seed and much statistical manipulation. Squillace (1957) showed that seed weight differences resulting from controlled pollinations are often caused by the tendency for "clustering" of female flowers used for each pollen on a given seed tree, and by poor cone yields. Seed weight effects can be compensated for statistically by adjustment techniques as used in the present study, but experimental control is preferable over statistical control whenever possible. Large differences in seed weight can be avoided by using a kind of "randomized block" procedure suggested by Squillace (1957) and also by increasing the allowance for cone losses. This procedure will also minimize variation in mean cone length which in turn greatly affects seed yield, and eliminate bias in other respects.

Lacking definite segregating qualitative factors as "gene markers" the use of growth rate for estimating the degree of selfing vs. crossing in mixed pollinations seems to be fairly satisfactory. As a measure of growth rate in one-year-old seedlings the height above cotyledons seems to be preferable to total height because it is affected less by seed weight *(table 2)* and can be measured more accurately (not subject to inconsistencies in ground level determination). Cotyledon number may or may not be useful in selective fertilization tests. After adjusting cotyledon number for seed weight effect, it proved useful in two of the tests, while in a third there apparently was no transmissible difference between the particular parents used.

Summary

1. Three tests were made specifically to determine whether any selective fertilization effects occurred when pollen of a given western white pine was in competition with that of another tree of the same species. In each test, matings were made between two trees, A and B, as follows: A×A, A×B, and A×(A+B). In the latter type of mating, pollens from the two parents (A+B) were mixed in equal proportions. Differences in early growth rate (length of epicotyl and total height) among the resulting one-year-old progenies from matings within tests were used as the main basis for estimating the degree of selective fertilization. Other factors such as mean cotyledon number and seed yield had limited usefulness.

2. In a moderately self-fertile tree crossing exceeded selfing. In a highly self-fertile tree, crossing exceeded selfing when its own pollen was mixed with that from one tree, while the reverse was true when its own pollen was mixed with that of another tree.

3. Results of the tests, along with less reliable but more extensive determinations of the growth rate of wind-pollinated vs. self- and cross-pollinated seedlings produced earlier, suggest that in stands under natural pollination conditions crossing largely exceeds selfing.

4. Additional testing is needed to verify results obtained and, further, to test the amount of selfing that may be expected under seed orchard conditions. Here, especially when limited numbers of clones each with many members are used, the potentiality for selfing may be magnified. Some suggestions of technique for future selective fertilization tests are included.

Zusammenfassung

Titel der Arbeit: *Selektive Befruchtung bei Pinus monticola Dougl. — I. Vorläufige Ergebnisse. —*

1. Es wurden drei Versuche durchgeführt, um zu ergründen, ob selektive Befruchtungserscheinungen vorkommen, wenn Pollen einer bestimmten "Western White Pine" mit dem eines anderen Baumes derselben Art in Wettbewerb steht. In jedem Test führte man folgende Paarungen von je 2 Bäumen (A und B) durch: A×A, A×B und A×(A+B). Für die zuletzt genannte Kombination wurde Pollen zweier Eltern (A+B) zu gleichen Teilen vermischt. Als Grundlage für die Bestimmung des Grades der selektiven Befruchtung benutzte man Unterschiede im Wachstum (Länge des Epikotyls und Gesamthöhe) zwischen den einjährigen Nachkommenschaften dieser Kombinationen. Auch andere Merkmale, wie mittlere Kotyledonenzahl und Samengehalt waren begrenzt brauchbar.

2. Bei einem mäßig selbstfertilen Baum wird die Selbstung von der Fremdung übertroffen. Bei einem im hohen Maße selbstfertilen Baum übertrifft die Kreuzung die Selbstung, wenn der eigene Pollen mit dem eines anderen Baumes gemischt wurde, mischte man ihn mit dem Pollen eines anderen Baumes, trat das Gegenteil ein.

3. Die Resultate dieser Untersuchungen weisen zusammen mit den weniger verläßlichen aber ausgedehnteren Wachstumsvergleichen zwischen den aus Windbestäubung, Selbstung und Fremdung hervorgegangenen Sämlingen darauf hin, daß die Kreuzung in Beständen mit natürlichen Pollenflugverhältnissen die Selbstung wesentlich übertrifft.

4. Um die vorliegenden Resultate zu bestätigen und um den unter Plantagenbedingungen zu erwartenden Selbstungsanteil zu prüfen, sind weitere Untersuchungen notwendig. Besonders, wenn eine begrenzte Klonzahl mit jeweils zahlreichen Pfropflingen verwendet wird, dürfte die Möglichkeit zur Selbstbestäubung steigen. Für zukünftige Untersuchungen über selektive Befruchtung werden einige technische Vorschläge gemacht.

Résumé

Titre de l'article: *Fertilisation sélective chez Pinus monticola Dougl. — I. Premiers résultats. —*

1. Trois essais furent entrepris pour déterminer si une fertilisation sélective se produit lorsque le pollen d'un *Pinus monticola* donné est en concurrence avec celui d'un autre arbre de la même espèce. Dans chaque essai, le croisement fut réalisé entre deux arbres, A et B, comme suit: A×A, A×B et A×(A+B). Dans le dernier type de croisement, les pollens des deux parents (A+B) étaient mêlés en proportions égales. Le degré de fertilisation sélective fut estimé parmi les descendants d'un an d'après les différences constatées dans la croissance juvénile (longueur de l'épicotyle et hauteur totale). D'autres facteurs, tels que le nombre moyen des cotylédons et la production de graines, ont un intérêt plus limité.

2. Chez un arbre modérément auto-fertile, l'hétérofécondation l'emporte sur l'autofécondation. Chez un arbre

fortement auto-fertile, on obtient le même résultat quand le pollen de cet arbre est mélangé avec celui d'un certain arbre, tandis qu'on obtient le résultat inverse lorsque le mélange se fait avec le pollen d'un autre arbre.

3. Les résultats d'essais moins précis, mais de portée plus générale, portant sur la vitesse de croissance de semis résultant de pollinisation libre, comparée à celle de semis résultant d'autofécondation et de croisement contrôlé, suggèrent que, dans les conditions de peuplements soumis à la pollinisation naturelle, l'hétéro-fécondation excède largement l'autofécondation.

4. De nouveaux essais sont nécessaires pour vérifier les résultats obtenus et aussi pour déterminer le taux d'autofécondation qui peut se produire dans un verger à graines. Dans ce cas, spécialement lorsqu'on utilise un petit nombre de clones reproduits chacun à un grand nombre d'exemplaires, les possibilités d'autofécondation peuvent être nettement accrues. Les auteurs font des propositions pour de nouvelles techniques de fertilisation sélective.

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). — BUCHHOLZ, JOHN T.: Origin of cleavage polyembryony in conifers. Bot. Gaz. **81**, 55—71 (1926). — EAST, E. M.: Studies on self-sterility. IV. Selective fertilization. Genetics **4**, 346—355 (1919). — JENSEN, HOLGAR: Inavelsrisken vid skogsfröplantager. Svenska Skogsvårdsföreningens Tidskr. (Stockholm) **43**, 178—180 (1945). — JONES, DONALD F.: Selective fertilization in pollen mixtures. Biol. Bull. **38**, 251—289 (1920). — JONES, DONALD F.: Selective fertilization. Univ. Chicago Press, Chicago, Illinois. 163 pp. (1928). — LANGNER, WOLFGANG: Die Klonanordnung in Samenplantagen. Z. Forstgenetik **2**, 119—121 (1953). — LANGNER, WOLFGANG, und STERN, KLAUS: Versuchstechnische Probleme bei der Anlage von Klonplantagen. Z. Forstgenetik **4**, 81—88 1955). — MOFFETT, A. A.: Genetical studies in Acacias I. The estimation of natural crossing in black wattle. Heredity **10**, 57—67 (1956). — ORR-EWING, A. L.: Controlled pollination techniques for Douglas-fir. Forest Sci. **2**, 251—257 (1956). — PHILP, JAMES, and SHERRY, S. P.: The degree of natural crossing in green wattle, *Acacia decurrens* WILLD. and its bearing on wattle breeding. Jour. So. Afric. For. Assoc. **14**, 1—28 (1946). — PIATNITSKY, S. S.: (On pollination in oaks and the germination of pollen on the stigmas.) Dokl. Akad. Nauk. S.S.S.R. **56**: 545—547 (1947). — Forestry Abstr. **9**, 1452 (1948). — SQUILLACE, A. E.: Variation in cone properties, seed yield, and seed weight in western white pine when pollination is controlled. Montana State Univ. School of Forestry, Bull. **5**, 16 pp. (1957). — STOCKWELL, W. P.: Preembryonic selection in the pines. Jour. Forestry **37**, 541—543 (1939).

Buchbesprechungen

Statistische Methoden für landwirtschaftliche Versuche. Von A. MUDRA. Verlag Paul Parey, Berlin und Hamburg, 1958. 336 Seiten, Preis 58,60 DM.

Das jetzt vorliegende Buch ist keine Neubearbeitung der beiden früher (1949 und 1952) im Verlag S. Hirzel erschienenen des gleichen Verfassers, sondern nach Anlage und Inhalt gänzlich anders zu bewerten. Auch ist es nicht allein auf Feldversuchstechniken beschränkt wie seine Vorgänger, sondern stellt eine Einführung in die wichtigsten statistischen Verfahren des landwirtschaftlichen Versuchswesens überhaupt dar. Im ersten Teil werden die statistischen Grundlagen in leichtverständlicher Weise behandelt, wie überhaupt auf Leichtverständlichkeit größter Wert gelegt wurde. Die Prinzipien der Probenahme, Herleitung und Eigenschaften der gebräuchlichsten Statistiken, die wichtigsten Verteilungen sowie die einschlägigen Rechenverfahren sind übersichtlich behandelt und mit vielen, gutausgewählten Beispielen belegt. Der zweite Teil ist der Planung und Auswertung von Versuchen gewidmet. Im Mittelpunkt dieses zweiten Teils stehen natürlich die Versuchspläne für den Feldversuch, aber es werden auch Fütterungsversuche und dgl. gebracht. Eine Anzahl, meist aus dem bekannten Buch von COCHRAN und COX entnommener Pläne werden dem Leser die Verrechnung der Gitteranlagen sehr erleichtern, zumal für jedes der beschriebenen Gitter ein Modellbeispiel in vorbildlich übersichtlicher Form durchgerechnet wurde. Im dritten Teil schließlich werden „Sonderfälle“ behandelt, wie die Ergänzung fehlender Teilstückerträge, Normalitätsprüfungen und Transformationen. Der Tabellenanhang enthält die Normalverteilung, Verteilung für t und F, für χ^2 sowie für den Korrelationskoeffizienten; neben der Transformation für r in z wurde auch die Transformation in arc sin $\sqrt{\%}$ angegeben, und schließlich erleichtern eine Tabelle zufallsmäßiger Zahlenfolgen, eine Tafel für Quadratzahlen und Quadratwurzeln und eine Tafel dreistelliger dekadischer Logarithmen die Planung und Auswertung.

Referent möchte aber doch auch auf einige nicht unbedenkliche Seiten des Buches hinweisen: Es ist selbst dann, wenn es ausschließlich für den Versuchsansteller und Studenten der Landwirtschaft bestimmt ist, ein unbedingter Nachteil, daß die elementaren logischen Operationen, auf denen die statistischen Verfahren aufgebaut sind und die zu ihrem Verständnis unbedingte Voraussetzung sind, nicht klar genug herausgestellt wurden. Hieraus ergeben sich gewisse Unschärfen. Zwei Beispiele mögen dies zeigen: Auf Seite 7, Abs. 1.4, heißt es: „Beurteilung der Stichprobe durch Prüfung auf Signifikanz. Diese läuft im wesentlichen darauf hinaus, daß entweder von der bekannten Grundgesamtheit auf die Stichprobe geschlossen wird (direkter oder Inklusionsschluß) oder umgekehrt, von der Stichprobe auf die unbekannte Grundgesamtheit (indirekter oder Repräsentationsschluß).“ Auf Seite 50, Abs. 6.1, wird das Ziel der Signifikanztests wie folgt definiert: „Der Zweck der statistischen Prüfung ist, zu entscheiden, ob die Nullhypothese richtig oder falsch ist. . . . Den Ausdrücken ‚wesentlich‘ oder ‚unwesentlich‘ werden wir von nun an sehr oft begegnen, sie bedeuten in der Statistik etwa dasselbe wie die Worte ‚schuldig‘ oder ‚nichtschuldig‘ in der Rechtsprechung. . . . Manche Autoren bevorzugen allerdings den Ausdruck ‚statistische Sicherheit‘ statt ‚Signifikanz‘.“ Man kann sich nur schwer vorstellen, daß der unbefangene Leser hier ein zutreffendes Bild gewinnt, zumal der Eindruck entsteht, als identifiziere der Verfasser „Test einer Hypothese“ (hier der Nullhypothese) und „Signifikanztest“. (Aber vgl. hier die Definition von „signifikant“, „Hochsignifikant“ usw. auf Seite 50!) Ein einfacher Rückgriff auf die Grundbegriffe der Wahrscheinlichkeitstheorie hätte hier wahrscheinlich mehr Klarheit gebracht. Schließlich vermißt man jeglichen Hinweis auf die Methode der kleinsten Quadrate, dies besonders im Hinblick auf die Zweckbestimmung des Buches als Lehrbuch für Studierende. Auch die Diskussion der Versuchspläne wäre hierdurch im einzelnen verständlicher geworden, so etwa die des Lateinischen Quadrats, dessen Pläne Abb. 25 b und c in so großem Widerspruch zum „Modell“ des Lat. Quadrates stehen, daß sie eigentlich nicht mehr hierhergehören.

Aber das mag den Wert des Buches nicht wesentlich vermindern, zumal der Verfasser im Vorwort selbst bemerkt, daß es nicht die Aufgabe des Buches sei, das Studium der ausführlichen statistischen Werke zu ersetzen. In diesem Sinne gebraucht, wird es dem bereits im Forschungsbetrieb tätigen manche Anregung vermitteln, vor allem aber wird er auch von der umfassenden Erfahrung des Verfassers in der Versuchsanstellung profitieren.

Der Verlag hat das mit dauerhaftem Leineneinband versehene Werk hervorragend ausgestattet, insbesondere sind Satz und Druck der reichlich vorkommenden mathematischen Gleichungen, Tabellen usw. vorbildlich. STERN

Genetisches und Cytogenetisches Wörterbuch. Von R. RIEGER und A. MICHAELIS. Zweite erweiterte Auflage. Mit 149 Abbildungen. IV, 648 Seiten. Springer-Verlag, Berlin-Göttingen-Heidelberg, 1958. Ganzleinen 49,60 DM.

Die erste Auflage des vorliegenden Wörterbuches war 1954 als Sonderheft des „Züchter“ erschienen (vgl. Besprechung in Z. Forstgenetik 4, 65, 1955). Die Bedeutung der Herausgabe eines derartigen Nachschlagewerkes auch für die Disziplinen der Forstgenetik und Forstpflanzenzüchtung wurde bereits damals gewür-