Selective Fertilization in Pinus nionticola Dougl.

II. Results of Additional Tests

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Inbreeding and concomitant depression of growth, a peril inherent in tree seed orchards, has received substanlial thought and speculation during the past decade. A solution to the problem has been sought through schemes of systematic clone arrangement, mathematical estimates of the number of clones needed to avoid serious inbreeding effects, and studies of pollen dispersion and selective fertilization[2]). Through studies of selective fertilization we hope to learn if inbreeding is mitigated by a selective discrimination against self-pollen, *per se*. Our investigations show no such discrimination against self-pollen in completely self-fertile trees.[3]) When self- and outcross-pollens were in competition in such trees, either selfing or outcrossing might yield a significantly greater number of germinable seed. One factor responsible for the outcome may be pollen vigor, which may reflect the vigor of the pollen parent. In partially self-fertile trees, outcross pollens were consistently more effective than self-pollen in yielding germinable seed.

In our initial study of selective fertilization (SQUILLACE and BINGIIAM, 1958) the general concepts of selective fertilization, inbreeding, and self-fertility, a literature revicw, and experimental procedures were presented. In design, our second study of selective fertilization essentially follows the previous experiment with the refinements suggested in the 1958 paper. Therefore, the present paper explains only changes in experimental design and improvements or modifications in experimental procedures, presents results, and discusses their significance for seed orchard management.

Experimental Procedure, Basic Data, and Results

Four test trees were chosen as seed parents. Tree 58 was chosen because of its complete self-fertility, and also because we wished to repeat the test of this tree previously described by SQUILLACE and BINGHAM (1958). Tree 64 was Selected because it was partially self-fertile and because albino seedlings were known to occur in its selfed progenies (addendum to BINGHAM and SQUILLACE, 1955). Trees 54 and 69 were selected because of their known self-fertility (54 partially self-fertile and 69 completely self-fertile), and because they grow at a relatively high elevation (*circa* 4,800 feet). Since flowering time of these trees is about 10 days later than that of trees at lower elevations, the problem of collecting pollens in time for use was alleviated.

The four female parents were chosen partly because of their self-fertilizing ability, and this factor proved vital to the analysis of results of the experiment. The relative self-fertility of each tree was judged on (1) amount of sound seed per cone after selfing and outcrossing (*table 1*), (2) number of seedlings surviving after 100 days to 1 year in the nursery[4]) (*table* 2), and (3) germinability in self- vs. cross-pollinations and the relative yield of self-pollinated seedlings (BINGHAM and SQUILLACE, 1955).

The sound seed yield following self-fertilization of trees 54 and 64 is consistently lower than the yield following outcrossing (*table* 1). The number of seedlings surviving the first year after sowing is markedly lower after selfing than after outcrossing (*table* 2, and BINGHAM and SQUILLACE, 1955). Therefore, we consider trees 54 and 64 "partially self-fertile." In self-fertilizing ability, these tres are similar to the majority of western white pine trees for which we have seed yield data over a period of several years.

In contrast, trees 58 and 69 consistently yielded as many, or more, sound seed per cone from selfing as from outcrossing (*table* 1). For tree 69, seedling survival in self-pollinated lots averages the same as in cross-pollinated lots (*table* 2, and BINGHAM and SQUILLACE, 1955). The survival of seedlings from self-pollinations of tree 58 was higher than from cross-pollinated lots. Therefore, we have termed these trees "completely self-fertile."

Table 1. — Sound seed yield after outcrossing and selfing.

	Seed parent	Seed year	Outcrossing		Selfing
			crosses	Sound seed yield per cone[1])	Sound seed yield per cone[2])
			Number	*Number*	*Number*
Completely self-fertile trees	58	1951	11	106	134
		1955	2	78	89
		1958	3	104	104
		Average		96	109
	69	1953	3	84	X7
		1958	2	120	118
		Average		102	102
Partially self-fertile trees	54	1951	4	106	80
		1952	3	110	102
		1958	2	157	110
		Average		124	97
	64	1953	—	—	13
		1954	4	56	6
		1958	3	182	71
		Average		119	30

[1]) Averages for all outcrosses made during the year shown.
[2]) Average for the self-mating for the year shown.

By "partially self-fertile" we mean trees that normally set fewer viable seeds per cone when selfed than when outcrossed, and whose seed germination and 1-year seedling survival are less in selfing than in outcrossing. This category could be further subdivided into moderate self-fertility, low self-fertility, and other gradations between self-infer-

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[2]) In this study we use the term "selective fertilization" in its broadest sense, i. e. "all types of discrimination in reproduction" (JONES, 1928), including selection before, during, and after fertilization (as evidenced at the end of the first growing season).

[3]) By "completely self-fertile" we mean trees that normally set as many viable seeds per cone when selfed as when outcrossed, and whose seedling survival in selfed lots equals that in outcross lots.

[4]) The survival count for this study was taken 1 year after sowing rather than at the end of the first growing season because germination in the second spring following spring sowing is not uncommon in western white pine.

Table 2. — Survival in June 1960 of selfed and check tree progenies from seeds of four trees sown May 1959.

Parents ♀ × ♂	Seed sown May 1959	Seedlings alive June 1960	
	Number	*Number*	*Percent*
58 × 58	900	595	66
1 × 58[1]) 2 × 58 3 × 58	2,700	1,425	53
69 × 69	900	334	37
1 × 69 2 × 69 3 × 69	2,700	1,187	44
64 × 64	900	144	16
1 × 64 2 × 64 3 × 64	2,700	1,422	53
54 × 54	900	144	16
1 × 54 2 × 54 3 × 54	2,700	1,222	45

[1]) Trees 1, 2, and 3 are check trees and were selected and mated to check the potency of the various pollens used in the selective fertilization tests. Data from the three outcrosses made with each pollen are combined.

tile (self-sterile) and completely self-fertile. The terms "completely self-fertile" and "partially self-fertile" are arbitrary, since the difference between them is only one of degree. Distinction between the two pairs of trees, 58 and 69, and 54 and 64, is important in this study, however, because the pairs apparently differ markedly in their degree of self-fertility. For trees 58 and 69 there is, apparently, very little reproductive discrimination against selfing, while trees 54 and 64 show a relatively high degree of discrimination.

Pollen was collected from trees 58, 69, 54, 64, and two additional sources — tree 18 and mm. Pollen termed mm (multiple mix) was a mixture of equal volumes of pollen

Table 3. — Controlled pollinations made in 1957.

Seed parent	Pollen parents							
					[1]) 1:1	[1]) 1:1	[1]) 1:1	[2]) 9:1
58	58	18			58 + 18			
69	69	64		mm	69 + 64		69 + mm	69 + mm
54	54	69		mm	54 + 69		54 + mm	54 + mm
64	64	18	58	mm	64 + 18	64 + 58	64 + mm	

[1]) Self-pollen plus an equal part of outcross pollen.
[2]) Nine parts self-pollen plus one part multiple-mix pollen.

from 8 trees: 17, 18, 21, 22, 25, 58, 59, and 62. We used this multimix pollen to simulate conditions in a seed orchard where strobili might receive pollen from several neighboring clones. Controlled pollinations using these pollens were made during the early summer of 1957 *(table 3)*.

To test fertilizing ability, three "check" trees were pollinated with each pollen. The various pollens were applied at random to strobili in all trees to minimize variation in seed yield and seed weight among crosses within each test (Squillace, 1957; Squillace and Bingham, 1958). Thus, we attempted to avoid clustering of strobili pollinated by one pollen in any given portion of the crown.

In the fall of 1958, cones were collected, seeds were extracted, and sound and hollow seeds were separated and counted. A sample of each seed lot, i. e. 58 × 58, 58 × 18, etc., was weighed *(table 4)*. Seeds were stratified for 120 days at 35° F. They were sown in three completely randomized blocks in the nursery at the Northern Idaho Forest Genetics Center, Moscow, Idaho, in May 1959. Three hundred seeds of each lot were sown in each of the three randomized blocks, with but three exceptions.[5])

Colored toothpicks, different colors representing successive germination periods, were set beside seedlings germi-

[5]) Lot 54 × 69 — 190 seeds per block; 64 × (64 + mm) — 209 seeds per block; check tree No. 3 × 18 — 220 seeds per block.

Table 4. — Results of controlled pollinations using self-pollen and a variety of outcross pollens in varying proportions

	Parents	Flow. polli-nated	Cones ma-tured	Blind seed per cone	Sound seed Total	Sound seed Per cone		Sound seed Weight	1—22 Days	22—28 Days	28—35 Days	35—42 Days	Over 42 Days
					Number		*Percent*[1])	*Mg.*	*Percent*				
Completely self-fertile	58×58	29	28	19.8	[2]) 2,894	103.4	83.9	18.2	65.3	29.8	4.6	0.3	—
	58×18	32	28	3.6	[2]) 3,391	121.1	97.1	19.1	83.3	11.0	4.4	1.3	—
	58×(58+18)	28	21	10.7	[2]) 2,639	125.7	92.1	19.5	67.7	22.0	9.3	1.0	—
	69×69	15	10	88.0	[2]) 1,185	118.5	57.4	15.5	28.3	35.7	12.3	4.0	19.7
	69×64	15	8	4.8	1,025	128.1	96.4	17.4	30.0	39.9	19.8	0.4	9.9
	69×(69+64)	19	15	29.5	[2]) 1,987	132.5	81.8	15.2	21.8	36.2	15.6	3.8	22.6
	69×mm	16	14	10.4	[2]) 1,575	112.5	91.5	14.3	35.0	37.0	17.7	4.3	6.0
	69×(69+mm)	15	13	31.4	1,459	112.2	78.1	14.2	39.3	42.0	17.0	1.7	—
	[3]) 69×(69+mm, 9:1)	15	13	65.7	1,015	78.1	54.3	14.6	21.3	30.0	19.8	2.7	26.2
Partially self-fertile	54×54	13	10	80.6	1,101	110.1	57.7	17.9	9.1	20.9	16.4	4.5	49.1
	54×69	13	5	86.6	569	113.8	56.8	19.8	24.8	32.9	13.4	5.4	23.5
	54×(54+69)	16	10	60.1	[2]) 1,911	191.1	76.1	17.5	35.3	23.5	13.2	4.5	23.5
	54×mm	20	19	11.7	[2]) 3,783	199.1	94.4	19.0	36.0	26.7	23.3	3.3	10.7
	54×(54+mm)	19	16	36.6	[2]) 2,817	176.1	82.8	18.5	40.0	30.4	21.7	2.2	5.7
	[3]) 54×(54+mm, 9:1)	15	15	75.4	[2]) 2,537	169.1	69.2	17.7	11.6	24.2	26.8	6.3	31.1
	64×64	15	14	121.3	990	70.7	36.8	18.7	32.0	30.6	14.9	0.7	21.8
	64×18	12	12	5.9	[2]) 2,413	201.1	97.1	17.7	44.1	33.4	20.4	2.0	—
	64×(64+18)	11	9	40.1	1,277	141.9	78.0	17.3	31.6	41.4	21.5	3.3	2.2
	64×58	14	12	5.2	[2]) 2,210	184.2	97.2	18.6	44.0	36.6	17.7	1.7	—
	64×(64+58)	12	10	30.0	[2]) 1,649	164.9	84.6	18.4	47.3	37.0	12.3	3.4	—
	64×mm	10	9	5.3	[2]) 1,459	162.1	96.8	18.2	40.8	35.1	19.7	4.4	—
	64×(64+mm)	11	3	52.7	627	209.0	79.9	18.0	29.1	43.3	20.7	1.5	5.4

[1]) Sound seed expressed as a percentage of total (sound plus blind) seed.
[2]) Total number of seeds estimated by weight.
[3]) Nine parts self-pollen to one part multimix pollen; in all other pollinations the ratio of self to outcross pollen is 1:1.

Table 5. — Germination and survival of progenies.

Parents ♀ × ♂	Seeds: Sown 1959	Seeds: Germinated 1959[1]		Seeds: Germinated 1960	Living seedlings June 1, 1960	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
[2]1 × 58 2 × 58 3 × 58	2,700	1,708	63.2	93	1,425	52.8
64 × 58	900	—	—	1	528	58.7
[2]1 × 69 2 × 69 3 × 69	2,700	1,570	58.1	95	1,187	44.0
54 × 69	569	—	—	1	149	26.2
[2]1 × 54 2 × 54 3 × 54	2,700	1,256	46.5	154	1,222	45.3
[2]1 × 64 2 × 64 3 × 64	2,700	1,678	62.1	141	1,422	52.7
69 × 64	900	—	—	2	284	31.6
[2]1 × 18 2 × 18 3 × 18	2,461	1,871	76.1	7	1,415	57.5
[3]58 × 18 64 × 18	1,800	—	—	5	929	51.6
[2]1 × mm 2 × mm 3 × mm	2,700	1,986	73.6	71	1,657	61.4
[4]69 × mm 54 × mm 64 × mm	2,700	—	—	2	1,198	44.4

[1]) Based on count of living seedlings 9/28/59.
[2]) Sum of three lots having the same male parent.
[3]) Average of two lots having 18 as male parent, 58 × 18 and 64 × 18.
[4]) Average of three lots having mm as male parent, 69 × mm, 54 × mm, and 64 × mm.

nating 22, 28, 35, and 42 days after sowing *(table 4)*. In September 1959 the total height and epicotyl length (from cotyledons upward) were measured to the nearest millimeter, and the number of cotyledons was counted for a maximum of 100 seedlings per block. The maximum number of seedlings measured per lot was 300.

In June 1960 all living seedlings in each check lot were counted. Germination in 1959 and delayed germination in 1960 and 1-year survival data for check lots and a corresponding lot (same pollen parent) were used to assess the effectiveness of self- and outcross-pollens *(table 5)*. No major differences in fertilizing ability of outcross pollens 58, 69, 64, 54, 18, and mm were found, although the fertilizing ability of pollens from 54 and 69 (both high elevation trees) appeared to be somewhat lower than that of the others tested. Pollen germination *in vitro* was not determined, since it is not pollen germinability but sound seed and seedling yield that is important in determining major differences in vitality existing between pollens.

Selective Fertilization Tests and Discussion of Results

A series of eight tests was made to compare (1) self-pollination — A × A, (2) outcross pollination — A × B, and (3) self-plus outcross-pollination — A × (A + B). In each three-pollination test, epicotyl length, germination time, and cotyledon number were compared by a t-test. For seed parent 58 there was only one three-pollination test (test 1). Seed parents 69 and 54 each had two tests (2 and 3, 4 and 5, respectively); seed parent 64 had three tests (6, 7, and 8).

Epicotyl length and cotyledon number for each lot were adjusted for seed weight by an average regression coefficient derived from the 1958 study. A regression of mean epicotyl length on germination time was computed for full-sibs in each block of each progeny. The regression coefficient was nonsignificant or so low in practically all instances that we felt no adjustment of epicotyl length for germination time was warranted. In this experiment, then, epicotyl length and germination time are essentially independent measures of pollen effectiveness when full-sib lots are compared.

The reasoning behind the data analysis follows this pattern: Self-fertilized seedlings grow slowly and have short epicotyls as compared with cross-fertilized seedlings. That is, a seedling with a short epicotyl is probably self fertilized, while a seedling with a long epicotyl is probably from a cross-fertilization. Therefore, statistical tests of differences between the mean epicotyl length of the three matings were used to determine the amount of self- or cross-fertilization.

Completely Self-fertile Tree (58 and 69)
Seed Parent 58 — Test 1

Test 1 mates trees 58 and 18 and is identical to test 2 reported in the 1958 article. In that study progenies of the 58 × 18 cross were only 13 percent taller than seedlings from the cross 58 × 58. In another experiment, however, the cross 58 × 18 gave seedlings averaging 36 percent taller than seedlings from 58 × 58. Thinking that perhaps pollens had been mislabeled in the initial study, we repeated this test in its entirety. Now we know that the cross 58 × 18 produced epicotyl length markedly superior to the self *(table 6)*. When pollens of 58 and 18 were mixed, mean

Table 6. — Comparison of basic data for progenies of seed parent 58.

Mating	Parents ♀	Parents ♂	Mean epicotyl length	Mean germination time	Cotyledons	Seedling basis
Number	*Number*		*mm*	*Days*	*Number*	*Number*
1	58	58	17.3	24.5	7.6	302
2	58	18	24.6	23.5	7.6	300
3	58	58+18	19.5	24.7	7.7	300
Statistical significance between matings[1])			1 and 2 = ** 1 and 3 = **	1 and 2 = ** 1 and 3 = NS	1 and 2 = NS 1 and 3 = NS	

[1]) ** = highly significant ($P < 0.01$); NS = nonsignificant ($P > 0.05$).

epicotyl length was only slightly greater than that from the selfed lot. Thus, it is probable that the lots 58 × 18 and 58 × (58 + 18) were inadvertently exchanged in test 2 of the initial experiment.

On the basis of epicotyl length in both the 1958 and present experiments, pollen from 58 was more effective in yielding germinable seed than pollen from 18. The ratio of self-fertilization to cross-fertilization is 70 : 30, both in the present study and in the same test described in the 1958 article.

Outcrosses of 58 to 18 germinated significantly faster than selfs ($P < 0.01$). When the pollen mix (58 + 18) was used, germination time was not significantly different ($P = 0.4$) from the selfed lot. This lends additional support to the finding that 58 pollen is more effective than 18 when both are competing to fertilize seed parent 58. There was no significant difference between the average cotyledon number of the three lots ($P > 0.05$). Seed soundness and seed weight data *(table 4)*, however, indicate a discrimination against selfing. From test 1 we conclude that when equal quantities

Table 7. — Comparison of basic data for progenies of seed parent 69.

Test	Mating	Parents ♀	Parents ♂	Mean epicotyl length	Mean germination time	Cotyledons	Seedling basis
		Number		mm	Days	Number	Number
2 and 3	1	69	69	14.9	33.2	7.7	300
2	2	69	64	15.2	30.4	7.7	253
	3	69	69 + 64	15.3	34.6	7.8	257
3	4	69	mm	17.8	29.4	7.6	300
	5	69	69 + mm (1:1)	18.1	27.1	7.8	300
	6	69	69 + mm (9:1)	14.1	35.8	7.6	263
Statistical significance between matings[1]				1 and 2 = NS 1 and 3 = NS 1 and 4 = ** 1 and 5 = ** 1 and 6 = NS 4 and 5 = NS	1 and 2 = ** 1 and 3 = NS 1 and 4 = ** 1 and 5 = ** 1 and 6 = ** 4 and 5 = **	1 and 2 = NS 1 and 3 = NS 1 and 6 = NS	

[1]) ** = highly significant ($P < 0.01$); NS = nonsignificant ($P > 0.05$).

of self-pollen and outcross-pollen are applied simultaneously to a completely self-fertile tree, self-pollen produces more germinable seeds.

Seed Parent 69 — Test 2 and 3

Progeny from the outcross 69 × 64 showed no statistically significant difference ($P = 0.4$) in epicotyl length from the selfed lot, 69 × 69 *(table 7)*. Therefore, we cannot use epicotyl length to determine the relative effectiveness of either 69 or 64 pollen when they are in competition. There is, however, a statistically significant decrease in germination time when the outcross progeny are compared to selfed progeny *(table 7)*. Average germination time for the mixed mating, 69 + 64, did not differ significantly from that of the selfed lot, indicating that more selfed plants (69 × 69) were produced than outcross plants (69 × 64). When pollens 69 and 64 are in competition, in female strobili of 69, more fertilizations are accomplished by 69 than by 64. Negligible differences in the number of cotyledons were observed. Although data on seed soundness *(table 4)* indicate discrimination against selfing, data on seed weight point to a discrimination against outcrossing.

In contrast to the 69 × 64 outcross, progeny of the 69 × mm outcross had significantly longer epicotyls than the selfed progeny ($P < 0.01$). When pollens 69 and mm were competing in a ratio 1 : 1, mm pollen was more effective *(table 7)*. However, when nine times as much 69 pollen as multimix pollen was used, the mean epicotyl length of the progeny dropped sharply. In this instance, because of the large volume of 69 pollen, the eggs were fertilized mainly by self-pollen.

Data on germination time, cotyledon number, seed weight, and seed soundness bear out the finding that for seed parent 69 self-pollen was not as effective as multimix pollen when in competition in equal amounts. For seed parent 69, when self-pollen was competing with 64 pollen alone, the self-pollen apparently fertilized more eggs, but when many pollens were available, the outcross pollen was favored. The reason why selfing is apparently favored in one instance and not in the other is discussed in a separate section below.

Partially Self-fertile Trees (54 and 64)

Seed Parent 54 — Tests 4 and 5

When pollens 69 or mm are in independent competition with 54 pollen, the mean epicotyl length of the resulting progenies is significantly greater and mean germination time significantly less than that of seedlings from the selfed progeny of tree 54 *(table 8)*. Thus outcross pollens are more effective in yielding germinable seed than self-pollen. This outcome is probably due to the relatively low self-fertility of tree 54. For example, even if 54 pollen fertilized more eggs than 69 pollen, many resulting embryos might not have developed because of self-incompatibility barriers, whereas embryos from more of the eggs fertilized by 69 pollen would develop, germinate, and grow. When the ratio of self-pollen to mm pollen is 9:1, however, self-pollen is significantly more effective: the amount of outcross pollen

Table 8. — Comparison of basic data for progenies of seed parent 54.

Test	Mating	Parents ♀	Parents ♂	Mean epicotyl length	Mean germination time	Cotyledons	Seedling basis
		Number		mm	Days	Number	Number
4 and 5	1	54	54	9.1	43.0	7.6	110
4	2	54	69	14.8	34.8	7.5	149
	3	54	54+69	13.6	34.0	7.8	221
5	4	54	mm	16.7	30.9	7.7	300
	5	54	54 + mm (1:1)	16.2	29.0	7.9	230
	6	54	54 + mm (9:1)	10.0	38.8	7.7	190
Statistical significance between matings[1]				1 and 2 = ** 1 and 3 = ** 2 and 3 = NS 1 and 4 = ** 1 and 5 = ** 4 and 5 = NS 2 and 4 = ** 1 and 6 = NS	1 and 2 = ** 2 and 3 = NS 1 and 4 = ** 4 and 5 = * 2 and 4 = ** 1 and 6 = **	1 and 2 = NS 1 and 3 = ** 1 and 4 = NS 1 and 6 = NS	

[1]) ** = highly significant ($P < 0.01$); * = significant ($P < 0.05$); NS = nonsignificant ($P > 0.05$).

Table 9. — Comparison of basic data for progenies of seed parent 64.

Test	Mating	Parents ♀	Parents ♂	Mean epicotyl length	Mean germination time	Cotyledons	Seedling basis
	Number			*mm*	*Days*	*Number*	*Number*
6, 7, 8	1	64	64	11.7	33.3	7.8	147
6	2	64	18	19.0	27.0	7.7	300
	3	64	64+18	18.7	28.7	7.7	275
7	4	64	58	17.7	26.8	7.6	300
	5	64	64+58	19.7	26.5	7.5	300
8	6	64	mm	20.1	27.5	7.7	300
	7	64	64+mm	15.1	29.4	7.7	203
Statistical significance between matings[1])				1 and 2 = ** 1 and 3 = ** 2 and 3 = NS 1 and 4 = ** 1 and 5 = ** 1 and 6 = ** 4 and 5 = ** 3 and 5 = NS 1 and 7 = **	1 and 2 = ** 1 and 3 = ** 2 and 3 = ** 1 and 4 = ** 1 and 5 = ** 3 and 5 = ** 4 and 5 = NS 1 and 6 = ** 1 and 7 = ** 6 and 7 = **	1 and 2 = NS 1 and 3 = NS 1 and 4 = ** 1 and 5 = ** 4 and 5 = NS 1 and 6 = NS 1 and 7 = NS	

[1]) ** = highly significant ($P < 0.01$); NS = nonsignificant ($P > 0.05$).

is probably too small to have noticeable effect. No significant differences were found between numbers of cotyledons for self- and outcross-progenies.

Seed Parent 64 — Tests 6, 7, and 8

The present study confirms the findings of Bingham and Squillace (1955) that tree 64 is only partially self-fertile, besides showing that albino seedlings are produced in the ratio of three green to one albino when the tree is selfed. When outcross pollen from tree 18 or 58 is in competition with 64 pollen, the mean epicotyl length of the resulting seedlings is significantly greater, and the mean germination time is significantly less than that for self-pollen (*table 9*). In these instances, therefore, outcross pollen is apparently more effective in yielding germinable seed than self-pollen. Seed soundness and seed weight data trends both agree with this finding (*table 4*). There was no significant difference in number of cotyledons between 64 × 64, 64 × 18, and 64 × (64 + 18) progenies. However, 64 × 58 progeny had significantly fewer cotyledons than 64 × 64 progeny. Because there are no significant differences in cotyledon number between 64 × 58 and 64 × (64 + 58) progenies, we have additional evidence that 58 pollen is more effective than 64 pollen when the two are in competition. We would expect 58 and 18 pollens to be more effective than self-pollen because tree 64 is only partially self-fertile. The data confirm this expectation.

When mm pollen is competing with 64 pollen, however, self-pollen is favored slightly but not significantly. The difference between the mean epicotyl length when 64 and mm pollen were in competition (15.1 millimeters) and the expected mean had there been equal action of the two pollens (15.9 millimeters) is not significantly different as shown by chi-square analysis. Germination time, seed soundness, and seed weight trends indicate that outcross pollen is favored.

The occurrence of albino seedlings when tree 64 is selfed provides a marker that is more reliable than differences in epicotyl length and germination time for evaluating the effectiveness of self- and outcross-pollen. The chlorophyll-deficient seedlings were white or ivory-colored with a pink cast (*figure 1*). Seedling color varied from flamingo pink to whitish pink with a faint green cast in the hypocotyl. The hypocotyl usually exhibited a more intense pink cast than

Table 10. — Expected and actual percents of albino seedlings and selfing occurring in progenies of tree 64.

Parents ♀	Parents ♂	Expected albino	Actual albino	Expected selfing	Actual selfing
		Percent			
64	64	25.0	25.3	100	100
64	64+18	12.5	5.7	50	23
64	64+58	12.5	4.3	50	17
64	64+mm	12.5	4.8	50	19

the cotyledons. We did not observe the wide variety of chlorophyll mutant types described by Eiche (1955) for *Pinus sylvestris*. The mutant seedlings resulting from self-fertilization of tree 64 occurred in the ratio three green to one albino ($P = 0.89$) (*table 10*), and were apparently all of one discrete class. We assume, therefore, that the albino trait is controlled by a single recessive gene, and that gametes carrying this gene and those not possessing this gene possess equal ability to combine and produce viable embryos. The albino seedlings lived for 2 or 3 weeks and died after the food in the endosperm was exhausted.

Since we obtained 25 percent albinos with 100 percent selfing, we would expect 12.5 percent albino plants if 64 and a competing pollen in a 1 : 1 mix were equally effective in yielding germinable seed. In the three tests of this hypothesis, however, we discovered only about 5 percent albinos (*table 10*). Assuming that this 5 percent represents one-fourth of all selfed individuals, the total of selfs in each test is approximately 20 percent. Clearly, pollen of 64 is less effective than the competing pollens when both are in competition in strobili of tree 64.

Relation of Parent Tree Vigor to Pollen-tube Vigor

Completely Self-fertile Trees

Self-pollen of highly self-fertile tree 69 was in competition with two outcross pollens. Self-pollen was more effective in producing germinable seed in test 2, and outcross pollen was more effective in test 3 (*table 7*). The data, though meager (tests 1, 2, and 3), indicate that discrimination against self-pollen does not always occur in completely self-fertile trees. There could be many differences in pollens which might explain why one pollen was more effective than another pollen when both are competing to fertilize the same egg, e. g., differences in viabilty due to

Figure 1. — Albino (upper bed) and typical green (upper and lower beds) western white pine seedlings.

proper or improper collection and handling, differential ability of pollen, though viable, to germinate on the nucellus, differential growth rate of the pollen tubes in the nucellus, ability of pollen tube and contents to live in and adapt to the nucellus for approximately 12 months, liberation of the generative nucleus, ability to penetrate the embryo sac, and specific incompatibility between the sperm and egg resulting in the death of the zygote at fertilization or at some point thereafter. In angiosperms, pollen germination and pollen-tube growth have received much consideration, and specific self-incompatibility systems have been described (LEWIS, 1954; BATEMAN, 1954; JONES, 1928). Pollens probably differ somewhat in respect to all these factors. Can our data be explained largely on the basis of one factor or a combination of several related factors?

Let us assume that general pollen-tube vigor is responsible for the observed differences. This vigor could embrace (1) rapid germination, (2) rapid pollen-tube growth, (3) ability to live in and adapt itself to the nucellus, and (4) ability to penetrate the embryo sac and fertilize the egg. Might not the general vigor of the germ cells reflect the genetic vigor of the parent tree itself? MARCET (1950) reported that the mean pollen-tube length of six Scotch pine trees paralleled their respective mean total height. A correlation coefficient of 0.706 ($P = 0.05$) was computed from MARCET's data. Although encouraging, these data in no way suggest that a high correlation between pollen-tube growth and parent tree vigor is extant within a given population.

To further evaluate the above hypothesis the mean periodic annual increment, adjusted for locality and age, of 44 candidate trees was taken from unpublished data at the Northern Idaho Forest Genetics Center.[6] The trees were then ranked according to their mean periodic annual increment from 1 to 44 and the ranking converted to a percentile. The vigor ratings (percentile rank) of the trees in this experiment are: 58 = 93, 54 = 89, 18 = 75, mm = 58, 69 = 34, and 64 = 2.

If we assume that these vigor ratings also apply to the tree's pollen, in test 1 we would expect pollen from 58 to be more effective than that from 18 when the two are in competition. In test 2, we would expect 69 pollen to be more effective than 64 pollen, and in test 3 mm pollen to be more effective than 69. The data show this to be true in each test.

[6]) A description of the measurement technique and an evaluation of the data are being prepared for publication.

Partially Self-fertile Trees

For completely self-fertile trees 58 and 69 the effectiveness of pollens when in competition is explainable on the basis of a positive, phenotypic correlation of pollen vigor with parent tree vigor. Is there an analogous situation for the two partially self-fertile trees? Because of the discrimination against selfing known to exist in these trees, it is meaningless to compare self-pollens with outcross-pollens. We can, however, compare two outcross pollens that were tested in competition with the same self-pollen. Two outcross pollens, 58 and 18, were tested in competition with self-pollen of tree 64. The vigor rating of tree 58 is 93; the vigor rating of tree 18 is 75. If tree vigor and pollen vigor are positively correlated, we would expect 58 pollen to be somewhat more effective than 18 pollen when in competition with 64 pollen. The data show that pollen of 58 is more effective than 18 pollen in increasing mean epicotyl length ($P < 0.08$) and reducing germination time ($P < 0.01$) when both are in independent competition with 64 pollen.

Two outcross pollens, 69 and mm, were tested in competition with self-pollen of tree 54. The vigor rating of tree 69 is 34, while the average rating for trees from which the mm pollen was collected is 58. The pollen from the parent having the higher vigor rating, mm, significantly increased epicotyl length ($P < 0.01$) and decreased germination time ($P < 0.01$) in comparison to the action of the pollen of less vigorous tree 69 when both were in independent competition with 54 pollen.

The data from completely self-fertile and partially self-fertile trees suggest a positive phenotypic correlation between parent vigor (mean periodic annual height growth) and pollen vigor. The ability to select the fastest growing western white pine trees by pollen-tube growth tests in the laboratory instead of cumbersome field measurements and estimates would greatly enhance the efficiency of our improvement program. The strength of this correlation (realizing that it may be 0) is being determined by analytical techniques. Until the phenotypic correlation is determined, we remain skeptical that the correlation exists or that it is high enough to merit a major change in our program. Even if the phenotypic correlation were high, such a technique could be successful only if the genetic correlation was high.

Implications for Seed Orchard Management

Our results suggest that the degree of selfing occurring in clonal seed orchards can be reduced by omitting completely self-fertile trees. Most western white pine trees are apparently partially self-fertile, while a few are completely self-fertile. The degree of self-fertility can be estimated by selfing each candidate selection and comparing seed yields against outcross seed yields. Culling of completely self-fertile candidates then may be warranted.

Results of the tests, on the other hand, still leave unanswered the question of the actual degree of selfing that may be expected in clonal orchards. Theoretically, because of wide spacing and the presence of ramets of the same clone within pollinating distance, the opportunities for selfing will be greater than in natural stands. Even in natural stands it seems probable that the relative quantity of self-pollen available to the female strobili of a given tree may greatly exceed the quantity of outcross pollen available to it. In conducting the experiments we had expected that outcross pollen would compete favorably against self-pollen, even when the latter was greatly in excess. But this did not occur. When self-pollen greatly exceeded outcross pollen (9:1) much selfing apparently occurred in both tests conducted for this purpose.

Thus, a complete solution to the question of inbreeding in clonal orchards will have to await results of studies of selfing under natural pollination conditions and refined pollen dispersion studies, possibly using radiological techniques or marker genes.

Summary

1. Eight tests involving four female parents and six male parents were made to determine the extent of reproductive discrimination between competing self- and outcross-pollens of western white pine trees. Each test comprised three matings between two trees, A and B, as follows: $A \times A$, $A \times B$, and $A \times (A + B)$. In all but two tests the ratio of competing pollens was 1:1; in two tests the ratio was 9 parts self to 1 part outcross pollen. In three pollinations a pollen mix composed of pollens from eight trees was used to simulate seed orchard conditions.

2. Two seed trees were termed "completely self-fertile," and two trees were termed "partially self-fertile" on the basis of sound seed per cone and seedling survival. Differences in epicotyl length at the end of the first growing season and the average time of germination of each lot were used as independent measures to estimate the degree of selfing and outcrossing.

3. In one completely self-fertile tree selfing exceeded outcrossing when competing pollens were in the ratio 1:1. In the second completely self-fertile tree selfing predominated when self-pollen was in competition with pollen of one tree (ratio 1:1), but outcrossing exceeded selfing when self-pollen was in competition with the pollen mix (ratio 1:1). Since selfing exceeded crossing in two of the three instances in the completely self-fertile trees, there is apparently no general discrimination that always favors outcrossing.

4. In the two partially self-fertile trees, when outcross and self-pollens were competing in the ratio 1 : 1, outcross pollen was more effective in yielding germinable seed than self-pollen in practically every test. .

5. In one completely self-fertile and one partially self-fertile tree the relative effectiveness of competing self- and outcross-pollens was assessed when the ratio was 9:1. In both tests the more abundant self-pollen was more effective in yielding germinable seed.

6. Albino seedlings in the ratio 1 white to 3 green were found to occur in the selfed progeny of partially self-fertile tree 64. The occurrence of this qualitative characteristic allowed a more precise evaluation of the relative effectiveness of self- and outcross-pollens. Results of pollinations of strobili of tree 64 with pollen from two other trees and the pollen mix show that in each test outcross pollen was five times as effective in yielding germinable seeds.

7. A positive correlation between parent tree growth rate and pollen-tube vigor was advanced and discussed as an explanation for the relative effectiveness of different pollens when in competition with the same self-pollen. Our findings could be explained by this relationship in nearly every instance.

8. Since most western white pine trees we have tested show a moderate to strong discrimination against self-pollen, we may expect a relatively small amount of germinable seed resulting from self-pollinations in a seed orchard. The danger of inbreeding and accompanying growth depression

can be reduced further by excluding completely self-fertile trees from the seed orchard.

Zusammenfassung

Titel der Arbeit: *Selektive Befruchtung bei Pinus monticola Dougl. II. Die Ergebnisse weiterer Versuche.*

1. Mit 4 Müttern und 6 Vätern wurden 8 Bestäubungstests durchgeführt, um das Ausmaß unterschiedlicher Befruchtungsfähigkeit zwischen konkurrierenden Selbstungs- und Fremdungspollen bei *Pinus monticola* festzustellen. Jeder Test umfaßte die 3 folgenden Kombinationen zwischen zwei Bäumen A und B: $A \times A$, $A \times B$ und $A \times (A + B)$. Mit zwei Ausnahmen betrug in allen Tests das Mischungsverhältnis zwischen den beiden Pollensorten 1:1; in den Ausnahmefällen betrug es 9 Teile Selbstungspollen : 1 Teil Fremdpollen. 3 Bestäubungen wurden mit einem Mischpollen von 8 Bäumen ausgeführt, um den Verhältnissen in Samenplantagen nahe zu kommen.

2. Auf der Grundlage der Anzahl Vollkörner pro Zapfen und der Höhe des Pflanzenprozentes wurden zwei der Mutterbäume als „komplett selbstfertil" und die beiden anderen als „partial selbstfertil" bezeichnet. Unterschiede in der Epikotyl-Länge am Ende der ersten Vegetationsperiode und in der mittleren Keimdauer jeder Probe wurden als unabhängige Maßstäbe für den Grad der Selbstung oder Fremdung benutzt.

3. Bei einem komplett selbstfertilen Mutterbaum wurde bei einem Pollenverhältnis 1 : 1 die Fremdung von der Selbstung übertroffen. Bei dem zweiten komplett selbstfertilen Baum war die Selbstung überlegen, wenn der Selbstungspollen mit nur einer Fremdpollensorte gemischt war (1 : 1); die Selbstung wurde jedoch von der Fremdung dann übertroffen, wenn der Selbstungspollen mit einer Fremdpollen-Mischung in Konkurrenz (1 : 1) gestanden hatte. Da die Selbstung bei den komplett selbstfertilen Bäumen in zwei von drei Fällen der Fremdung überlegen war, scheint es generell offenbar keine Fertilitätsunterschiede zu geben, die stets die Fremdung begünstigen würden.

4. Wenn Selbstungs- und Fremdpollen im Verhältnis 1 : 1 gemischt waren, dann war der Fremdpollen in Bezug auf die Menge des erhaltenen keimfähigen Samens praktisch in jedem Test wirkungsvoller als der Selbstungspollen.

5. Bei einem komplett selbstfertilen und einem partial selbstfertilen Mutterbaum schätzte man die relative Wirksamkeit konkurrierenden Selbstungs- und Fremdungspollens bei einem Mischungsverhältnis von 9 : 1 ab. In beiden Fällen erwies sich der in größerer Menge vorhandene Selbstungspollen für den Samenansatz als wirkungsvoller.

6. Es wurde gefunden, daß in der Selbstungsnachkommenschaft des partial selbstfertilen Baumes Nr. 64 Albino-Sämlinge im Verhältnis 1 weiß : 3 grün vorkamen. Kreuzungsergebnisse des Baumes Nr. 64 mit Pollen von zwei anderen Bäumen als Mischpollen ließen erkennen, daß in jeder dieser Kombinationen der Fremdpollen 5 mal wirkungsvoller war als der Selbstungspollen, und zwar hinsichtlich der Anzahl der erhaltenen keimfähigen Samen.

7. Eine positive Korrelation zwischen der Wüchsigkeit des Elternbaumes und der Vitalität des Pollenschlauches wurde erkannt und in der Diskussion als eine Folge der relativen Wirksamkeit verschiedener mit ein und demselben Selbstungspollen konkurrierender Pollensorten erklärt. Unsere Befunde konnten in beinahe jedem Beispiel mit dieser Bezugsetzung in Einklang gebracht werden.

8. Da die meisten bisher untersuchten Bäume von *Pinus monticola* mittlere bis strenge Selbstungsunverträglichkeit aufwiesen, dürfte in einer Samenplantage nur eine relativ geringe Menge keimfähigen Selbstungssaatgutes zu erwarten sein. Die Inzuchtgefahr und die damit verbundene Wuchsdepression kann durch die vollständige Ausschaltung selbstfertiler Klone aus der Samenplantage weiter vermindert werden.

Résumé

Titre de l'article: *Fécondation sélective chez le Pinus monticola Dougl. Résultats d'essais supplémentaires.*

1. Huit essais comprenant quatre parents femelles et six parents mâles ont été réalisés dans le but de déterminer la proportion de pollen du même arbre et de pollen étranger qui participe à la fécondation lorsque ces deux pollens sont mis en compétition. L'espèce étudiée est le Pin Blanc de l'Ouest (*Pinus monticola* Dougl.). Chaque essai comprenait trois croisements réalisés entre deux arbres A et B de la façon suivante: $A \times A$, $A \times B$ et $A \times (A + B)$. Dans tous les essais sauf deux, le rapport des pollens concurrents était 1 : 1; dans deux cas ce rapport était de 9 parties de pollen du même arbre pour une partie de pollen de l'autre arbre. Dans trois croisements un mélange composé de pollen de huit arbres a été utilisé de manière à stimuler les conditions du verger à graines.

2. En se basant sur le nombre de graines fertiles par cônes et sur la survie des semis, deux des arbres ont été déterminés comme "complètement autofertiles" et deux autres comme "partiellement autofertiles". Les différences dans la longueur de l'épicotyle à la fin de la première saison de végétation et le temps moyen de germination de chaque lot ont été utilisés come mesures indépendantes pour estimer le degré d'autofécondation et de fécondation croisée.

3. Chez un arbre complètement autofertile, l'autofécondation a prédominé lorsque le pollen de l'arbre était en concurrence avec celui d'un autre dans le rapport 1 : 1, mais la fécondation croisée a dominé lorsque le pollen de l'arbre était en concurrence avec le mélange de pollens dans le même rapport. Puisque l'autofécondation a dominé la fécondation croisée dans deux cas sur trois pour les arbres complètement autofertiles, il semble qu'il n'existe aucune discrimination d'ordre général qui favoriserait toujours la fécondation croisée.

4. Chez les deux arbres partiellement autofertiles, quand les deur types de pollen étaient en concurrence dans le rapport 1 : 1 le pollen étranger a donné plus de graines viables que le pollen de l'arbre dans pratiquement tous les cas.

5. Pour un arbre complètement autofertile et pour un partiellement autofertile, l'efficacité relative du même pollen et du pollen étranger fut mesurée pour leur rapport 9 : 1. Dans tous les cas le pollen du même arbre, plus abondant, a donné plus de graines viables.

6. Des semis albinos ont été trouvés dans le rapport de un blanc pour trois verts dans une descendance autofécondée de l'arbre partiellement autofertile numéro 64. La manifestation de ce caractère qualitatif a permis une évaluation plus précise de l'efficacité relative des deux types de pollen. Les résultats de la pollinisation des fleurs femelles de l'arbre numéro 64 avec du pollen de deux autres arbres et avec le mélange de pollens, montrent que dans chaque essais la fécondation croisée est cinq fois plus efficace.

7. On a avancé et discuté l'hypothèse d'une corrélation positive entre la vitesse de croissance du parent et la vigueur du tube pollinique pour expliquer l'efficacité relative de pollens différents lorsqu'ils sont en concurrence avec le même pollen de l'arbre mère. Cette relation pourrait expliquer nos résultats dans presque tous les cas.

8. La plupart des Pins que nous avons testés manifestent à l'encontre de leur propre pollen une discrimination faible à forte; nous pouvons donc nous attendre, dans les vergers à graines, à une quantité relativement faible de graines viables résultant d'autofécondation. Le danger de consanguinité et de la réduction de vigueur correspondantes peut être encore réduit en éliminant complètement du verger à graines les arbres complètement autofertiles.

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A Provenance Study of Jack Pine Seedlings

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Introduction

With the increasing use of planting as a method of forest regeneration, it is important to have information about the genetic quality of the materials used. In a species like jack pine (*Pinus banksiana* LAMB.) with a nearly transcontinental distribution (*Fig. 1*), the variation between different provenances is likely to be considerable. A number of investigations of such variability in jack pine have been reported. SCHANTZ-HANSEN and JENSEN (10) described a test of 32 provenances of jack pine from localities in the United States and Canada grown in plantations in Minnesota. Large differences in size and form of the plants were evident; northern provenances were slower growing than southern. STOECKELER and RUDOLF (12) in Wisconsin found that foliage of seedlings of the more northerly provenances showed increased tendency to turn bronze-coloured in the cold season. Also the height growth of 2-year-old seedlings was correlated directly with the number of degree-days (base 50° F, 10° C) of the locality of origin and inversely with latitude. HOLST and YEATMAN (5) found for Ontario provenances grown in a nursery near Chalk River, Ontario, that height growth of transplants was correlated with the length of the growing season (base 42° F, 5,5° C) and the mean temperature for June, July, and August of the locality of origin. VAARTAJA (13 and 14) using greenhouse tests found that in jack pine, among other species, the more northerly provenances showed a greater response to variations in photoperiodic treatments.

CRITCHFIELD (3) found substantial variation in lodgepole pine (*Pinus contorta* DOUGL.) of various origins. NIENSTAEDT and OLSON (8) working with eastern hemlock (*Tsuga canadensis* [L.] CARR.) have shown the usefulness of provenance tests of seedlings grown under controlled conditions in growth chambers.

The present report deals with an experiment conducted in growth chambers where the effect of photoperiod and nitrogen supply on seedlings of jack pine was studied. Physiological results, averaged for all provenances, have been reported (4); presently, variations between the provenances are dealt with.

Materials and Methods

The experiment has been described in a previous paper (4). Materials consisted of nine provenances of jack pine (*Pinus banksiana* LAMB.) and one of lodgepole pine or western jack pine (*P. contorta* var. *latifolia* ENGELM.). Approximate locations of the parent stands are shown in *Fig. 1*, and relevant data are given in *Table 1*.

Each seed-lot was part of a larger seed-lot collected by the Department of Forestry, Ottawa, Canada and currently being used by that organization for provenance studies.

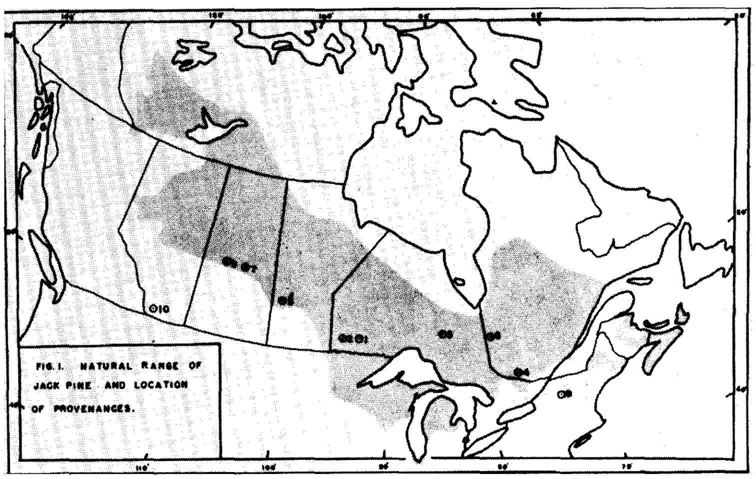

Fig. 1. — Map of Canada showing the range of jack pine and localities from which the seed were obtained. The numbers correspond with those in *Tables 1* and *2*. Based on RUDOLF (9).

The seedlings were grown for 115 days after sowing the seed. The root medium was a porous form of silica in steel-plated vessels 10 cm. tall and 6 cm. in diameter. The number of seedlings per vessel was reduced to five after germination was complete. The temperature was held near 20° C and relative humidity averaged about 75%. Light was provided by incandescent lamps; intensity was about

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