rank changes of family performance at different spacings. The significant interaction can be attributed to changes in trait variance at different spacings and is eliminated by an appropriate transformation. Test materials were 3-year-old seedlings of 30 families from a 5-males-within-6-females Design I mating grown at four spacings (1, 3, 5, and 7 inches apart) in a nursery.

Growth response of seedlings to spacings was subjectively compared (i. e., no statements of statistical reliability were possible) to responses in 43-year-old Douglas-fir and to responses in several crop and forage species. In terms of effect of spacing on relative variability, on shapes of frequency distributions, and on vegetative yield, seedlings and mature trees performed similarly to one another and to several agricultural species. On the assumption that similar responses to density imply similar types of genotype-spacing interaction, spacing-genotype interaction in Douglas-fir is not likely to appreciably affect selection accuracy, particularly if individual tree data are transformed to a scale which normalizes their distribution.

Key words: Douglas-fir, seedling growth, heritability, spacing-genotype interaction.

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Frequency of Seedlings from Natural Self-Fertilization in Coastal Douglas-fir

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Introduction

Because forest trees frequently become established in family groups, and because there appear to be no phenological or physiological barriers to self-fertilization or to cross-fertilization between related individuals, some inbreeding can be expected in the wind-pollinated progenies of naturally occurring trees. Published information about the natural inbreeding in conifers comes primarily from the genus Pinus.

Sarvas (1962) estimated that about 7% of all filled seed in Pinus *sylvestris* resulted from self-fertilizations and that self-pollination in the top one-third of the crown appeared to be only about 60% as frequent as in the lower two-thirds. He also reported that in more open mature pine stands, in seed tree stands, and in mixed stands, the share of self-fertilized full seed could rise to 10 to 20% of the total full-seed crop.

Squillace and Kraus (1963) estimated the degree of natural selfing in Pinus elliottii var. elliottii. They defined natural selfing as the production of viable seedlings by self-fertilization and obtained natural selfing estimates ranging from 0 to 27% for 11 trees. Nine of the trees showed 5% or less selfing, and two showed 23 and 27%, respectively.

FRANKLIN (1968) reported the percent of self-fertilized seedlings in wind-pollinated progeny of 25 marker-carrying trees in an old-field stand of *Pinus taeda* to be 2.4% for the 1966 seed collection and 1.1% for the 1967 collection. Estimates for individual trees ranged from 0 to 13.5% natural selfing (FRANKLIN 1968, *Table 13*). FOWLER (1965) listed the percent self-fertilization for three *Pinus banksiana* trees. It was estimated that 12.8% of the seeds obtained from the upper crown and 25.9% of the seeds from the lower crown of these 15-foot-tall trees resulted from self-fertilization.

With the exception of *Pinus banksiana*, these reports indicate that the frequency of seedlings from natural self-fertilization averages less than 10%, although certain trees producing considerably higher numbers of self seedlings have also been identified. Other estimates of natural selfing higher than 10% in conifers have also been published (see literature citations in FOWLER (1965), FRANKLIN (1968), SQUILLACE and KRAUS (1963)), but these were usually based on one or two trees per species.

Coastal Douglas-fir (*Pseudotsuga menziesii* var. *viridis*) produces mainly empty seeds following controlled self-fertilization (ISTRATOVA, 1964; ORR-EWING, 1954, 1957; SORENSEN, 1971 b). Natural self-fertilization should similarly produce mainly empty seeds and an average low level of inbreeding in open-pollinated progenies. However, there is also a small proportion of trees which set approximately one-fifth to one-half as many filled seeds after selfing as after crossing. The presence of these individuals, the almost complete intermingling of male and female strobili within the crown, and a type of stand regeneration which can readily lead to family groups, suggest that high levels of natural inbreeding are possible for some Douglas-fir progenies and perhaps for some stands.

The purpose of this paper is to estimate the frequency of self, or inbred, seedlings in open-pollinated progenies of Douglas-fir, to estimate the associated inbreeding depression and to discuss factors which influenced family variation in the amount of natural self-fertilization.

Materials and Methods

Description of Materials

The estimate of the frequency of self-pollinated seedlings was obtained for 19 Douglas-fir trees carrying recessive mutants or marker genes. The trees were located in 13 different stands at elevations ranging from 150 to approximately 5,000 feet in the Coast and Cascade Ranges of western Oregon. They came from a variety of situations, including closed and open, one- and two-storied, and pure and mixed stands. They also included trees which varied greatly in self fertility, as shown by comparison of seed yields after controlled self- and cross-pollinations.

With one exception, all trees were readily climbable, and cone collections were made by climbing. All collections were made in the top one-half to one-third of the crown. Collections were made in 1965, 1966, 1968, and 1970, with the bulk of the seeds collected in 1968. Seeds from some of the trees were collected in more than 1 year. Yield of filled seeds per cone was also recorded.

Nine of the mutant carriers were identified from controlled self-pollinations and 10 from germination tests of open-pollinated single tree seed collections. The latter 10 mutant carriers were found during the screening of approximately 700 open-pollinated seed collections using germination tests of 25 to 200 cleaned seeds. Tests of this size are often not large enough to identify mutant-carrying seed trees. Consequently, mutant carriers identified from germination tests of open-pollinated seed lots will likely be biased toward trees which set larger numbers of filled seeds after self-fertilization. This possibility will be discussed later.

Open-pollinated seed lots were germinated and seedlings maintained either in petri dishes in the germinator where white seedling mutants were involved or in vermiculite-filled sandwich boxes in growth chambers for all other mutants until they had reached the stage at which they could be separated into normal and aberrant classes. Mutant traits were such that all aberrant seedlings could be easily identified provided they were not affected by damping-off fungi, germination irregularities, or accidents. Approximately 2% of the germinant seedlings were affected by these factors and were not included in the tallies.

Because seeds were germinated in nearly ideal conditions, it may have led to a higher germination of mutants than would have occurred under more natural conditions (FRANKLIN, 1968). However, seeds from self-pollination and from open-pollinations were all germinated under the same conditions and at the same time. Consequently, germination of mutants should not have been favored in one class of seeds, for example, self-pollinated seeds, more than the other.

Estimation of Natural Selfing

For nine of the 19 tested trees, self-pollinated families were available in addition to the open-pollinated families. Ratios of total to mutant seedlings were determined in the self families, and the ratios were used to convert frequency of mutant seedlings in the open-pollinated family to frequency of self seedlings. For example, tree Marys Peak-4 had a ratio of 209 total to 68 mutant seedlings in the self family and, in 1968, 76 mutant seedlings among 14,457 seedlings in the open-pollinated family (*Table 1*). Number of self seedlings in the open-pollinated family of MP-4 was calculated as $76 \times (209/68) = 233.6$. Incidence of self seedlings in the open-pollinated family was estimated as $233.6/14{,}457 = 0.0162$.

The mean ratio of total-to-mutant seedlings for the nine self families was 5.1 : 1. This ratio was used to convert number of mutant seedlings to number of self seedlings in the 10 wind-pollinated families for which there was no corresponding inbred family. The 5.1 : 1 ratio seemed preferable to the theoretical total-to-mutant ratio of 4 : 1 with these families, because it was quite similar to a 4.9 : 1 average ratio reported for 33 segregating self families in loblolly pine (FRANKLIN, 1969), and because of other evidence for higher than expected ratios due to some pregermination selection against deleterious mutants (FOWLER, 1964).

As mentioned above, open-pollinated collections were made from some of the trees in more than 1 year. In this case, a percent self-fertilized seedlings was calculated for each year. An average value for the tree was obtained by weighting the percent self seedlings for each year by the seed yield (germinant seedlings/cone) for that year, which properly amplifies effects of good seed years.

All observations were made on seedlings. Because inbreeding in these progenies is recognized only by the presence of mutant seedlings, it is impossible to know whether it has been due to self-pollinations per se or to matings between other, presumably related[1]), trees carrying the same mutant allele. In the calculations, it has been assumed that each mutant seedling is *equivalent* to a

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[1]) A small number of mutants may occur due to matings between unrelated trees carrying the same mutant allele. This should be a very rare occurrence and has been ignored in subsequent calculations.

Table 1. — Estimated frequency of selfed seedlings in open-pollinated offspring of 19 western Oregon Douglas-fir trees.

Parent No.	Years of collection	Total No. of seedlings grown	Ratio of selfed/ mutant seedlings in true selfs	Apparent frequency of selfs in open pollinated families	
				Range1)	Average2)
	No.	No.	ratio	%	%
Elk Creek-6	3	1,533	5.2	0.0	0.0
Alsea-7	1	2,874	—	1.2	1.2
Hoskins-3	1	4,436	—	1.6	1.6
Marys Peak-2A	1	1,406	—	10.9	10.9
Marys Peak-4	2	15,121	3.1	1.6—4.7	2.4
Wards School-7	2	7,350	—	2.5—4.9	4.4
Berry Creek-10	1	2,245	—	13.6	13.6
Corvallis-8	3	6,962	6.3	2.2—3.9	2.8
Corvallis-F-11	2	4,817	5.3	17.8—49.5	27.5
Lacomb-2	2	6,060	4.9	17.8—18.0	17.9
Lacomb-10	1	3,752	7.3	0.0	0.0
Lacomb-11	1	221	5.3	2.4	2.4
McKenzie Bridge-11	1	1,154	—	1.8	1.8
Tombstone Pass-7	1	3,750	—	7.5	7.5
Fish Lake-21	1	2,558	—	7.6	7.6
Santiam Pass-1	1	8,275	3.7	19.4	19.4
Santiam Pass-12	1	5,870	—	9.1	9.1
Metolius-16	2	6,109	5.3	1.2—3.1	1.3
Metolius-29	1	5,072	—	1.7	1.7

1) Apparent selfing frequency for a tree was obtained by multiplying the frequency of mutant seedlings by the ratio of total/mutant seedlings for that tree (or by 5.1 if selfing data were not available).

2) Weighted averages based upon numbers of mutant seedlings and numbers of viable seeds per cone.

certain number of self seedlings independent of whether male parent was self, sibling, or other relative.

Results

Mutant Types

Four mutant phenotypes were recognized. Eight families segregated for normal seedlings and seedlings with yellow pigmentation. Actual shades of yellowness in the different families varied from light green to a very pale green or nearly yellow cotyledons. Seven additional families segregated for normal seedlings and seedlings with a "cotyledonary lethal" trait. When they initially appeared, these mutant seedlings ranged from normal in color to light green. However, shortly after shedding the seedcoat and before new primary needles were more than 1 mm long, the cotyledons became slate-grey-green in color. This was quickly followed by the cotyledons drying from the tip inward. This type of mutant has also been observed in ponderosa pine by the author and in loblolly pine (Franklin, 1969). Three families segregated for a "white seedling" marker and normal green seedlings (Sorensen, 1971 a). Finally, one family had mutant seedlings whose needles initially came out white, then slowly turned green after they were about 1 cm long.

It is likely that most of the mutant traits were controlled by single recessive genes. Therefore, the expected ratio in a self family was three normal seedlings for each mutant seedling. However, due to sampling error and other causes (Fowler, 1964; Sorensen, 1967), these ratios sometimes differed from 3 : 1, most often in the direction of higher ratios (Franklin, 1969; Rudolph, 1966; Snyder *et al.*, 1966). In the present test, segregation ratios in the selfed progenies ranged from 2.1 normal : 1 mutant to 6.3 normal : 1 mutant. Three ratios were significantly different from the expected 3 : 1. Two ratios had fewer than the expected number of mutant seedlings; one had more.

Estimation of Frequency of Self Seedlings

Estimated frequency of self seedlings in the open-pollinated progenies ranged from zero to 27.5% *(Table 1)*. Average for the 19 families was 7%. Eleven of the families had less than 5% natural selfing; three had more than 15%.

The mutant-carrying trees, which were identified through germination tests of open-pollinated seeds, gave a higher estimate of frequency of self seedlings (7.5%) than did the trees which were identified from their own self families (6.1%). The difference between the two groups was not significant, but it was logical because trees with very low frequencies of seedlings from self-fertilization (for example, Elk Creek-6 and Lacomb-10, *Table 1)* would probably not have been identified using germination tests of 25 to 200 open-pollinated seeds.

Estimates of the ratio of filled seed after self-pollination to filled seed after cross-pollination (self fertility) were available for nine of the seed trees (Sorensen, 1971 b), so the correlation between percent self seedlings in the open-pollinated families and self fertility was determined for these families. The correlation was low ($r = 0.343$) and not significant with 7 degrees of freedom.

Because controlled selfing in Douglas-fir usually results in a very greatly reduced seed set, it seemed likely that low yields of filled seeds in open-pollinated collections might in themselves be indicative of high frequencies of natural self-fertilization. This relationship was tested by correlating frequency of self seedlings in the open-pollinated families and seed yield in terms of germinant seedlings per cone. Correlation coefficient was —0.211 and nonsignificant. The relationship was also investigated by comparing frequency of self seedlings and seed yield in six trees which were tested in more than 1 year *(Table 1)*. For one tree the estimate of natural self-fertilization was zero. For four of the other five trees, seed yield and frequency of self seedlings were inversely related.

Discussion

Effect of Natural Self-fertilization on Growth

On the average, 7% of the seedlings in the newly germinated open-pollinated families could be considered as equivalent to self seedlings. Since a first generation self seedling would have an inbreeding coefficient (F) of 0.50, the 19 families reported here would have F = 0.035 relative to families which were produced from controlled crossings between unrelated parents.

Langner (1966) reported that for several plant species an increase in F of 0.10 is accompanied by a height growth reduction of about 5%. Applying that relationship to the Douglas-fir estimates, a height growth depression of 1.5 to 2% is obtained for the open-pollinated families. In other words, average stem height could be increased by about 1.5 to 2% by eliminating natural self-pollination.[2] If the boles of the trees can be treated as cone shaped and if diameter growth can be assumed to be affected by inbreeding to the same extent as height, then a volume increase of 4.5 to 6% might be expected by eliminating the inbreeding which accompanies wind pollination.

Because the inbred seedlings result both from self-pollination and from the exchange of pollen among related trees, some portion of this productivity increase should also occur by removing seed-producing trees from their natural habitat and putting them into orchards.

Frequency of self seedlings in the 19 open-pollinated progenies varied considerably *(Table 1)*. Eleven families had less than 5% self seedlings, with Elk Creek-6 and Lacomb-10 having no marker seedlings in the wind-pollinated families. At the other end of the scale, three families had over 15% self seedlings. When the frequency of self seedlings is high, the effect on growth could be quite drastic. For example, three trees have estimated frequencies of self seedlings of 17.9, 19.4, and 27.5. These figures would give F's of about 0.09, 0.10, and 0.14; estimated inbreeding depressions in height of 4.5, 5, and 7%; and estimated inbreeding depressions in stem volume of about 13, 14, and 20%, respectively.

Only a minority of open-pollinated progenies performed in this manner. However, this does mean that in evaluation tests of open-pollinated progenies, there will be downgrading of some parents for reasons other than their genetic worth. It also means that, if genetic variances are determined from a test of open-pollinated material, there will be family contribution to the variance which does not reflect the breeding value of the parentage (Namkoong, 1966).

Factors Affecting Frequency of Mutant Seedlings

Several factors can affect the frequency of mutant-bearing seedlings following open pollination. The frequency of selfing can be high if male and female flowers open at the same time within a tree and if nearby trees differ in flowering time. The frequency of selfing can also be high if a tree bears a heavy pollen crop, especially if it is in an open stand or in a mixed stand with few Douglas-fir.

But selfing itself may not result in many mutant-bearing seedlings. That can be the case if there is a high degree of self-sterility. When that happens, the selfing may be evident only from the numbers of empty seeds produced.

While I have interpreted a mutant-bearing seedling as evidence of selfing, other forms of inbreeding can give rise to mutant homozygotes. A tree may grow in a cluster of half sibs so that close relatives mate with each other. Or, in a 2-story stand a tree in the understory may be pollinated by its mother. In either case the frequency of mutant-bearing seedlings may be much larger than if there were complete panmixia.

Six of the 19 trees had apparent selfing frequencies, as measured by frequency of mutant types, greater than 9%. Of these:

Corvallis F-11 flowered later than its neighbors,

Santiam Pass-1 and S.P.-12 were understory trees possibly pollinated by one of their parents,

Berry Creek-10 and Marys Peak-2A were in pure even-aged stands.

Lacomb-2 was in a small group of trees which might be related. Thus, phenological differences may explain the selfing in one tree, mating among close relatives may have increased the apparent amount of selfing in two trees, and the high frequency of mutants remains unexplained in two.

Summary

The average frequency of seedlings from natural self-fertilization was estimated as 7% for 19 Douglas-fir trees growing in 14 different areas in western Oregon. The range was 0 to 27%. Eleven of the progenies had less than 5% self seedlings; three over 15%. Average inbreeding coefficient (F) was 0.035, where F = 0 for seedlings coming from controlled crosses between unrelated trees.

Assuming that each increase in F of 0.1 resulted in a height growth depression of 5%, the average inbreeding depression in height growth due to natural self-fertilization was estimated to be 1.5% to 2% and the depression in volume growth to be 4.5% to 6.0%.

Key words: Plant genetics, fertilization (plant), Douglas-fir.

Zusammenfassung

Die durchschnittliche Häufigkeit von Sämlingen aus natürlicher Selbstbefruchtung wurde mit 7% für 19 Douglasien aus 14 verschiedenen Gegenden in West-Oregon geschätzt. Es kamen 0% bis 27% vor. 11 der Nachkommenschaften hatten weniger als 5% Selbstungssämlinge, 3 Nachkommenschaften über 15%. Der durchschnittliche Inzuchtkoeffizient (F) war 0,035, wobei F = 0 für Sämlinge aus kontrollierten Kreuzungen zwischen nicht verwandten Bäumen angenommen worden war. — Bei der Annahme, daß jedes Ansteigen von F um 0,1 sich in einer Höhenwachstumsdepression von 5% äußert, wurde die durchschnittliche Inzuchtdepression im Höhenwachstum, die durch natürliche Selbstbefruchtung hervorgerufen wird, auf 1,5% bis 2% und die Depression im Dickenwachstum auf 4,5% bis 6,0% geschätzt.

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[2] This estimate does not take into account higher mortality among the inbred seedlings. For nursery grown stock, the early mortality and very weak cull seedlings would not reach the plantation and would not contribute to inbreeding depression in the field. On the other hand, higher mortality among inbred seedlings after outplanting would contribute more than expected to growth loss.

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Genetic variation in Saghalien fir from different areas of Hokkaido

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Introduction

Saghalien fir *(Abies sachalinensis)* is widely distributed throughout Hokkaido, the southern Kuriles and Saghalien. This fir is the most hardy among 5 species of *Abies* native to Japan and one of the most important forest tree in Hokkaido. In suitable habitats, it is also one of the most planted species for timber production. The selection of the best seed sources for planting is of economic interest.

Only a little information, e. g. Maruoka (1966) and Tamari (1965), is available so far on the geographic variations of Saghalien fir in Hokkaido. They observed the difference in early growth, the date of bud opening and the frequency of occurrence of secondary shoots between the seedlings from western and eastern parts of Hokkaido. This study was performed to determine the genetic variability of Saghalien fir among different areas in Hokkaido and to supply seed source information applicable to planting in different areas in Hokkaido.

Materials and Methods

In our studies we used Saghalien fir seedlings from natural forest sites of seven areas, including 117 individual mother trees. The number of trees from which seeds were collected and climatic and geographic data pertaining to the sites of their origin are given in *Table 1* and the locations of collection areas are shown in *Figure 1*. Each collection consists of seeds from 18 to 39 average mother trees from an area of several acres.

The seeds collected in September 1964 were sent to our Station. After measuring the length of 10 cones and the weight of 1000 seeds from each mother tree, the percent germination was averaged for three replicates of 100 seeds. The seeds were buried in snow for promoting germination during this winter.

On May 11 and 12, 1965, the seeds were sown in our nursery. As the number of seeds collected from each mother tree was different, the plot size of each mother tree's seed bed was different varying 100 cm^2 to 300 cm^2. Germination began from May 24 to May 30, and ended on June 10.

In the mid June, cotyledon number of seedlings was measured. At the end of the growing season, in mid September, 10 seedlings were dug up at random from the plots of each mother tree and site, taking care not to cut the roots. In the case of three sites, Nemuro, Sapporo and Rishiri, however, 60 seedlings were dug up. The height, root length (length of main root), diameter (at ground surface), dry weight of seedlings with shoots and roots (after drying at 110^0 C for 5 hours) and needle number (excluding cotyledon) were measured with one year seedlings.

In September, 1966, at the end of the second growing season, the seedlings were first transplanted. We sampled at random the seedlings from each plot and set up test plot by the method of split plot. The randomized block design with 4 replications was used. Each block consisted of seedlings from 117 mother trees from 4 sites and seedlings from mixed seed of mother trees from the other 3 sites. One plot for test of variance among mother trees included 64 seedlings per 1 m^2 and the plot for test of variance among sites using only mixed seed of mother trees included 786 seedlings in 12 m^2. After transplanting, the height and number of branches were measured in October, 1966, sampling 20 seedlings per plot. The height and diameter of 3-year seedlings were measured in September, 1967. The height and the frequency of occurrence of secondary shoots were measured in October, 1967 and 1968. We classified the Saghalien fir secondary shoots according to the method studied for Jack pine (Rudolph, 1964), namely (1) Lammas growth (developing terminal bud), (2) Prolepsis (developing laternal bud), (3) Lammas growth and Prolepsis. In the case of Saghalien fir, Prolepsis shoots are very frequent, and Lammas growth very few. We considered the mean value of the length of secondary shoots of 10 seedlings taken at random from each plot as a length of secondary shoot of each mother tree.

To clarify the geographical variation of Saghalien fir in Hokkaido, in eary March of 1969, one month before the

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