Electron spin resonance of irradiated DNA. J. Chem. Phys. 39: **839—840** (1963). — SPARROW, A. H., **ROGERS**, A. F., and SCHWEMMER, S. S.: Radiosensitivity studies with woody plants. I. Acute gamma irradiation survival data for 28 species and predictions for 190 species. Brookhaven Nat. Lab. Publ. 12116. 61 pp. (1968). — SQUIRES, T. L.: An introduction to electron spin resonance. Academic Press, New York. 140 pp. (1964). — STAIRS, G. R.: Effects of chronic and acute gamma irradiation of male flower buds and mature pollen in ***Quercus***. Forest Sci. *10:* **397—409** (1964). — STAIRS, G. R., and TROENDLE, V.: Male bud and pollen radiosensitivity in selected conifer species. Silvae Genetica (In Press, 1969). — TANOOKA, H.: Free radical concentration in irradiated bacterial spores and vegetative cells as measured by the ESR method. Rad. Res. 27: **570—581** (1966). — VIDAKOVIĆ, M.: Investigations on the intermediate type between the Austrian and Scots pine. Silvae Genetica 7: 12—19 (1958). — **VIDAKOVIĆ**, M.: Interspecific hybridization of several pine species from the sub-genus Diploxylon. In: World Consultation on Forest Genetics and Tree Improvement, Stockholm, FAO Forgen **63-2b/5**: 1—5 (1963). — WERTZ, J. E.: Nuclear and electronic spin magnetic resonance. Chem. Rev 55: 829—955 (1955). — WRIGHT, J. W., and GABRIEL, W. J.: Species hybridization in the hard pines, series ***Sylvestres***. Silvae Genetica 7: 109—115 (1958). — ZIMMER, K. G., and MÜLLER, A.: New light on radiation biology from electron spin resonance studies. In: Current Topics in Radiation Research, Vol. 1, M. EBERT and A. HOWARD, Eds., North-Holland Publ. Co., Amsterdam: 1—47 (1965).

---

# Estimate of Self-Fertility in Coastal Douglas-Fir from Inbreeding Studies

By FRANK SORENSEN[1])

## Introduction

Success of several methods of tree improvement can be influenced by the self-fertility of a species. The purpose of this paper is to present selfing results from Douglas-fir trees in western Oregon and to discuss those results in relation to management of seed orchards, to evaluation of open-pollinated progeny tests, and to development of homozygous lines.

Yield of filled seeds following self-pollination of Douglas-fir is reported to vary among trees from zero to about 20 filled seeds per cone[2]) (ALLEN, 1942; DUFFIELD, 1950; ISTRATOVA, 1964; ORR-EWING, 1954, 1956, 1957 a and b; WHEAT, 1965; SZIKLAI, 1966). One exceptional tree yielded as many filled seeds per cone after self- as after cross-pollination (ORR-EWING, 1957 b). There is also indication that average self-fertility of trees may vary among geographic areas (ORR-EWING, 1957 a).

Initial observations of self-fertility in Douglas-fir were made on isolated trees planted in Europe. These trees had low natural seed yield, and it was first suggested that this was because pollen shed occurred before female strobili on the same tree were receptive (LARSEN, 1937). Later, however, it was found that production of selfed seed was usually low even after controlled self-pollination (ALLEN, 1942; DUFFIELD, 1950). This indicated internal barriers to selfing.

ORR-EWING (1956, 1957 b) investigated this problem by cytologically studying ovule development after self- and cross-pollination. He observed that after selfing, pollen germination, syngamy and proembryo formation proceeded normally, but that embryos almost all collapsed soon after proembryo formation. From this it was concluded that embryo collapse was an inbreeding effect, caused by increased homozygosity of recessive lethal and deleterious genes.

The present investigation was undertaken to enlarge the sample of selfed Douglas-firs, partially because several of the above reports were based on planted trees of unknown or unidentified origin, and partially because all were based on relatively small numbers of trees.

Thirty-five Douglas-fir at five locations in western Oregon were self- and cross-pollinated in one or two of the years 1964, 1965, and 1966.

Seed yields were counted for both types of pollination. Relative self-fertilities were determined for each tree and expressed as the ratio of seed yield following selfing to seed yield following crossing.

Loads of deleterious genes responsible for the observed reduction in seed yield following self-pollination were calculated for each tree.

## Materials and Methods

Trees were selected for self- and cross-pollination in the following five localities on a west-east transect across the central Oregon Coast Ranges and the Cascade Mountains (*Figure* 1).

| Location | Longitude | Latitude | Elevation |
|---|---|---|---|
| | | | *(feet)* |
| Elk Creek | 123° 45' W | 44° 33' N | 150 |
| Marys Peak | 123° 30' W | 44° 30' N | 3,550 |
| Corvallis | 123° 15' W | 44° 39' N | 450 |
| Lacomb | 122° 42' W | 44° 35' N | 900 |
| Santiam Pass | 121° 48' W | 44° 25' N | 4,400 |

Elk Creek, Marys Peak, Corvallis, and Lacomb plots are in essentially continuous forests which are almost pure Douglas-fir. At Santiam Pass, Douglas-fir is a component of a mixed forest. The five locations bracket the main longitudinal range of coastal Douglas-fir in central Oregon.

Six trees were tested at each location with the exception of Lacomb, where 12 trees were selfed, and Marys Peak, where all cones on one of the six trees were lost to frost. Trees were selected solely on the basis of adequate crops of male and female strobili. Where possible, trees were separated by 200 feet or more, so closely related trees would probably not be sampled. However, the structure of the stands at Marys Peak and Santiam Pass did not permit this. Consequently, some of the tested trees within these two locations may be more closely related than at the other locations.

Pollination Techniques:

Female strobili were isolated in pollination bags before opening of floral bud scales. Male strobili were removed from twigs which carried female strobili isolated for cross-pollination; male strobili were left on twigs which bore female strobili isolated for selfing.

Two methods of self-pollination were used. One method, called "self-shake", was to shake the isolation bags covering male and female strobili on two or three different

---

[1]) Plant Geneticist, Forestry Sciences Laboratory, Pacific Northwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Corvallis, Oregon.

[2]) Maximum yield following cross-pollination ranges from about 50 to about 90 filled seeds per cone, depending on cone size, in the coastal form of Douglas-fir.

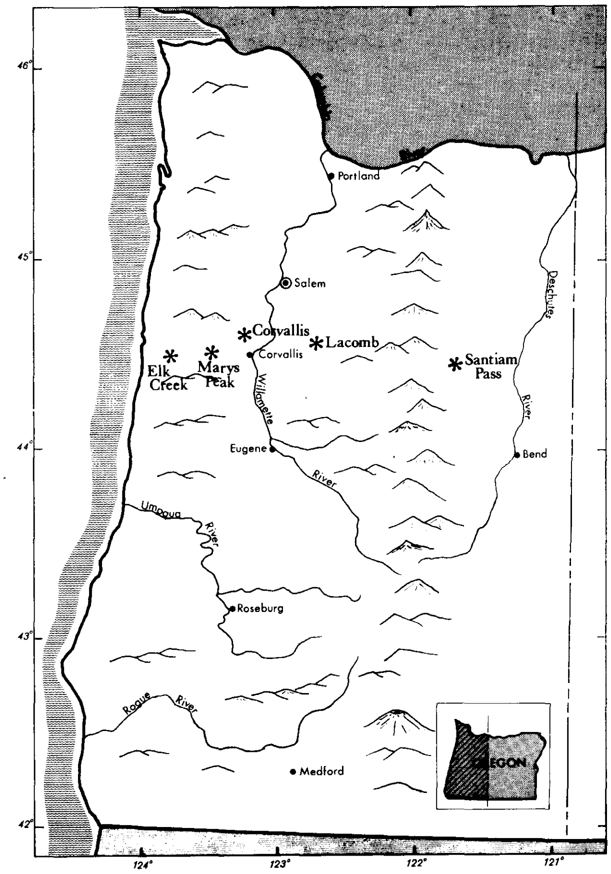

*Figure 1.* — Location in western Oregon of the five areas where Douglas-fir trees were self-pollinated.

days when pollen was shedding and female strobili were in or near the stage of maximum receptivity. The second method, called "self-pour", involved removing the isolation bag for approximately one to three minutes while carefully pouring self-pollen from a vial over each of the female strobili. All 35 trees were self-pollinated using the self-shake method. Twelve of the same trees were also self-pollinated using the self-pour method.

For the cross-pollinations, isolation bags were briefly removed and pollen poured over the female strobili. Pollen for crossing came from four to seven trees and was applied as a mix of approximately equal parts. Pollen trees were from the same geographic areas as the female trees, but were growing at least one-quarter mile away, so the probability of close relationship between seed trees and pollen trees should be small.

The objectives of the study required that both treatments (self and cross) should receive equivalent pollination. Three factors could influence the equivalence of pollination.

First, pollination techniques could have differed in effectiveness. As stated above, cross-pollen was poured from a vial on the female strobili, and self-pollen, in general, was applied by bagging male and female strobili together and shaking the bag when female strobili were receptive. Efficiency of the two techniques was checked during the 1964 pollination season by examining both self- and cross-pollinated strobili under a dissecting microscope. After pollen was poured on, it was observed in large amounts in the stigmatic areas throughout each strobilus. However, when pollen was shaken in the bag with the female strobili, it was observed that some stigmatic areas of some strobili had received very little or no pollen. Pollen techniques were not equivalent. To adjust for the inequality, 12 trees in 1966 were self-pollinated both by pouring the self-pollen on some female strobili and by shaking it with some female strobili. Yield of filled selfed seed after "shaking" was then related to yield after "pouring". For the remaining 23 trees, selfing was done only by "shaking". For these trees, yield of selfed seeds after "shaking" was adjusted to a yield after "pouring" by using the relationship developed from the trees selfed in both ways. Details of the relationship between the two selfing techniques are presented in the Results section.

Second, pollen may differ in viability. Only fresh pollen was poured on, and it was tested before use in a 0.15 percent $H_2O_2$ solution.[3] All lots tested 95 percent or higher in frequency of viable pollen grains. Samples of self-pollen grains which dropped into isolation bags were also tested and found to have 95 percent or higher viability. Therefore, pollen viability should not have differed between treatments.

Third, male strobili in the bags in which male and female strobili were enclosed together may not have shed their pollen when the female strobili on that tree were receptive; i. e., some trees may have been dichogamous. No markedly dichogamous trees were observed, and all isolation bags checked contained loose pollen at the time female strobili were receptive.

### *Seed Counts:*

Three to about 50 selfed and outcrossed cones were collected from each tree. Ten cones of each, if available, were chosen at random for seed counts. Cones were dissected by hand. Apparent full-sized seeds (called "round" seeds) and under-developed seeds (called "flat" seeds) were extracted, and total seed spots counted.

Self- and cross-pollinated cones had, on the average, the same proportion of round seeds to total seed spots on any one tree. Because fertilization in Douglas-fir takes place after the cone and potential seed have reached nearly full size (Allen, 1942), it was assumed that each round seed represented the potential for fertilization. On the other hand, it was observed that flat seeds were flat at approximately the time of fertilization and represented no potential for fertilization. Therefore, comparisons were based only on round seeds.

Round seeds were separated into filled and empty classes using a small laboratory seed separator (Silen, 1964) and the filled seeds counted. A cutting test of a large number of seeds showed that separation of filled and empty seeds was almost perfect.

### *Germination of Seeds:*

Seeds from both self and cross treatments were stratified and germinated in the spring of the year following collection. In some tests, seeds were sown in small pots containing a peat and loam mixture, and the pots placed in a glasshouse for germination. In other tests, seeds were germinated on filter paper in petri dishes in a germinator and set in the pots after germination. All seedlings were grown the first summer in a glasshouse.

## Results

### *Comparison of Seed Yields Using the Two Self-Pollination Methods:*

---

[3] This method, developed by Roy R. Silen, provides a quick test of Douglas-fir pollen viability.

*Table 1.* — Seed yields in filled seeds per cone after self-pollination using two methods of pollen application: pollen poured from vial onto female strobili, and pollen shaken in isolation bag with female strobili.

| Tree number | Method and year of pollen application and seed yield | | | |
|---|---|---|---|---|
| | Pollen poured on female strobili | Pollen shaken with female strobili | | |
| | *1966* | *1964* | *1965* | *1966* |
| EC-1 | 7.5 | | 8.2 | 0.8 |
| EC-2 | 1.2 | | 0.4 | 1.2 |
| MP-6 | 7.2 | | | 4.9 |
| C-8 | 2.7 | | | 3.1 |
| C-24 | 3.6 | | | 4.0 |
| C-29 | 10.5 | | | 10.4 |
| L-1 | 2.6 | 3.8 | | 2.1 |
| L-2 | 4.1 | 2.3 | | 3.7 |
| L-3 | 0.2 | 0.1 | | 0.1 |
| SP-1 | 18.9 | 10.1 | | 10.8 |
| SP-2 | 5.3 | 0.1 | | 6.7 |
| SP-3 | 0.1 | 0.08 | | 0.05 |
| Mean | 5.32 | | | 3.99 |

In general, the yield of filled selfed seeds was greater when self-pollen was poured on female strobili than when it was shaken together with female strobili in an isolation bag *(Table 1)*. Usually the difference in seed yield following the two methods of pollen application was not large. Sometimes, however, as with EC-1 in 1966, the yields were so different that serious misclassification of the self-fertility of a tree would have occurred if pollen was applied only by shaking.

Because the method of application of the cross-pollen was to pour it on the strobili, the comparison between self- and cross-fertility was based on this method of pollen application. For the 12 trees listed in *Table 1*, the comparison was made directly. For the 23 trees which were self-pollinated using the shaking technique only, selfed seed yields were adjusted before the comparisons of selfed and crossed seed yields were made. The adjustment used was the average ratio of selfed seed yield when pollen poured on strobili divided by selfed seed yield when pollen shaken with strobili (Model IA, p. 153, SNEDECOR, 1956). Only data for 1966 were used. Data for EC-1 were omitted from the calculations because they were far out of line with the other ratios. The average ratio was equal to 1.29.

*Yields of Selfed Seed:*

The pattern of seed yield following selfing conforms, in general, to that observed by ISTRATOVA (1964), ORR-EWING (1956), and SZIKLAI (1966) *(Table 2)*.

1. Average relative selfed seed yield was estimated to be 11.3 percent. That is, on the average, the trees set about 11 percent as many sound seeds after self-pollination as after controlled crossing. However, the distribution of relative self-fertilities is skewed toward lower percentages, so that the median tree has a relative selfed seed yield somewhat lower, or about 7.5 percent.

2. There was a wide range in selfing ability among individual trees. Estimates of self seed yields ranged from one or two seeds per 10 or more cones up to about 20 seeds per cone *(Table 1)*. Estimates of relative self-fertilities ranged from one-tenth of 1 percent to about 45 percent *(Table 2)*. Although none of these trees showed a self-fertility greater than 45 percent, ORR-EWING (1956) has reported one tree which set as many seeds after selfing as after crossing.

No trees proved self-sterile, although several gave very few selfed seeds. Such trees may or may not be self-sterile,

*Table 2.* — Yield of filled seeds after cross- and self-pollination.

| Location and tree number | Filled seeds per 100 round seeds after | | Relative yield of filled self-pollinated seeds |
|---|---|---|---|
| | Crossing | Selfing | |
| | | | (self÷cross) |
| Elk Creek | | | |
| 1 | 1)74.8 | 16.1 | 2)0.215 |
| 2 | 52.1 | 1.8 | 2)0.034 |
| 3 | 81.7 | 12.1 | 0.148 |
| 4 | 3)57.4 | 2.8 | 0.049 |
| 5 | 3)20.6 | 1.2 | 0.058 |
| 6 | 82.5 | 4)0.0 | 0.001 |
| Mean | 61.5 | 5.7 | 0.084 |
| Marys Peak | | | |
| 1 | 93.8 | 11.3 | 0.120 |
| 2 | 83.4 | 9.7 | 0.116 |
| 5 | 67.9 | 5)2.7 | 0.040 |
| 6 | 70.0 | 13.2 | 2)0.188 |
| 7 | 5)81.6 | 3)5.3 | 0.065 |
| Mean | 79.3 | 8.4 | 0.106 |
| Corvallis | | | |
| 8 | 80.6 | 8.0 | 2)0.099 |
| 24 | 90.6 | 6.2 | 2)0.068 |
| 25 | 48.4 | 4.6 | 0.095 |
| 26 | 65.6 | 4.0 | 0.061 |
| 28 | 43.6 | 3)3.3 | 0.076 |
| 29 | 75.5 | 17.0 | 2)0.225 |
| Mean | 67.4 | 7.2 | 0.104 |
| Lacomb | | | |
| 1 | 78.2 | 3.5 | 2)0.045 |
| 2 | 79.7 | 6.0 | 2)0.075 |
| 3 | 88.4 | 0.3 | 2)0.003 |
| 4 | 51.4 | 0.6 | 0.012 |
| 5 | 22.2 | 1.6 | 0.072 |
| 6 | 77.0 | 8.4 | 0.109 |
| 8 | 3)50.4 | 21.8 | 0.432 |
| 9 | 89.3 | 27.4 | 0.307 |
| 10 | 61.3 | 1.3 | 0.021 |
| 11 | 80.7 | 11.7 | 0.145 |
| 12 | 42.1 | 0.1 | 0.002 |
| 32 | 5)61.8 | 3)2.4 | 0.039 |
| Mean | 65.2 | 7.1 | 0.105 |
| Santiam Pass | | | |
| 1 | 67.2 | 31.2 | 2)0.464 |
| 2 | 62.0 | 17.4 | 2)0.281 |
| 3 | 83.6 | 0.6 | 2)0.007 |
| 4 | 78.7 | 3)8.6 | 0.109 |
| 7 | 88.8 | 6.1 | 0.069 |
| 8 | 3)89.7 | 3)8.2 | 0.091 |
| Mean | 78.3 | 12.0 | 0.170 |
| Grand mean | 69.2 | 7.9 | 0.113 |

1) Averages based on 10 or more dissected cones per treatment except where footnoted otherwise.

2) Self-pollinations on these trees made by pouring self-pollen on female strobili. Self-polinations on all other trees were made by "shaking" only. Yields of filled selfed seeds from the latter trees have been adjusted to make them comparable to seed yields when pollen was poured on the strobili. See text under results for further explanation of adjustment.

3) Average based on 5 to 9 cones.

4) No filled seeds found in first 10 dissected selfed cones, but extraction of remaining cones yielded one filled seed.

5) Average based on 3 to 4 cones.

because, where very few seeds are produced, the seeds could either be the result of self-pollination or apomixis (ALLEN, 1942; ORR-EWING, 1957 c). However, ORR-EWING[4]) has evidence that selfing tests should be quite extensive before self-fertility is assumed. For example, from one tree he obtained no seedlings from 505 seeds from a certain selfing in 1962. However, when selfed in 1965 it gave 38 seedlings from 3,639 seeds.

3. There was no significant difference among localities in average seed yield following selfing; the range in relative self-fertility among individual trees was wide at all locations *(Table 2)*.

4. Poor germination and postgermination mortality in $S_1$ (first generation, inbred families) families added relatively little to the loss described above. Average germination of filled selfed and crossed seed was approximately the same. However, postgermination lethal traits were found in several families *(Table 3)*. If those traits were due to simple recessives with normal transmission, a self family of 16 seedlings would offer a high probability ($P = 0.01$) of having a mutant seedling if the parent carried a mutant gene. Fifteen $S_1$ families included 16 or more seedlings, and of these, seven families, or about 40 percent of the families, showed presence of early postgermination lethal factors. Again, if we assume simple recessives and inheritance, these lethal traits further reduced the average relative self-fertility of 11.3 percent by approximately 10 percent, or about 10.3 percent (i. e., .90 × 11.3).

It should be pointed out, however, that germination and early growth were tested under favorable environmental conditions — generally in the greenhouse. In the field, where environmental stresses may be greater, germination of $S_1$ families might be relatively poorer and early postgermination mortality might be greater than was observed in the greenhouse.

## Discussion

Management of stands and seed orchards, evaluation of open-pollinated progeny tests, and success in the development of homozygous or partially homozygous lines can all be affected by the self-fertility of a species. Reduced self-fertility alone can affect success in obtaining inbred lines. However, for orchard management and progeny testing to be influenced, the species, in addition to having lower self- than cross-fertility, must also self-pollinate and yield filled selfed seeds under natural conditions, and the resulting selfed progeny must exhibit inbreeding depression. In coastal Douglas-fir there is evidence for both natural selfing[5]) and inbreeding depression (ORR-EWING, 1957 a; WHEAT, 1965), but the extent to which they occur has not been well established.

*Table 3.* — Description of lethal postgermination marker traits found as a result of the self-pollination.

| Tree number | Description of marker[1]) |
|---|---|
| EC-1 and L-11 | Pale green hypocotyl, normal cotyledons, short fine needles which are green on terminal 1—2 cm of epicotyl, but dry up below that. |
| L-1 | Short, delicate needles which die as they age, leaving only a tuft of green needles on end of stem. |
| L-2 and C-8 | Albino (lacks green color in hypocotyl and cotyledons; lacks yellow color in radicle; rapid germination). |
| L-10 | Green cotyledons, white needles initially which gradually turn green, especially along the midrib. |
| SP-1 | Pale green hypocotyl, normal cotyledons, no needles ever appear, seedling soon dies. |

[1]) Description of symptoms in greenhouse-grown seedlings.

### *Stand and Seed Orchard Management:*

The median tree in this survey was estimated to have a relative self-fertility of about 7.5 percent following controlled self-pollination. Of the 35 trees tested, 21 had an estimated relative self-fertility of less than 10 percent, and four trees had more than 25 percent. Thus, it appears that the majority of trees will not yield much selfed seed under either natural or seed orchard conditions even if there is a high proportion of natural self-pollination. The main result of any natural self-pollination will be reduced seed yield. However, that four of the 35 trees had estimated relative self-fertilities of near 30 percent or greater indicates that there is a potential for considerable natural production of selfed seedlings from some individuals (see also ORR-EWING, 1957 a). Since methods for identifying such parents are simple, such identification appears worth early attention in seed orchards.

### *Open-Pollinated Progeny Testing:*

Natural selfing and its potential consequence, inbreeding depression, are a concern because of the bias they may add to estimates of additive genetic variance based on open-pollinated seed (NAMKOONG, 1966). The observation that average self-fertility is quite low indicates that the portion of this bias introduced by selfing should also be low in coastal Douglas-fir. However, the fact that individuals are extremely variable in their self-fertility could make the importance of the bias depend upon the particular families sampled.

### *Directed Inbreeding:*

Until the amount of natural selfing and inbreeding depression are more precisely determined, the low self-fertility of Douglas-fir trees has greatest significance as it relates to prolonged inbreeding, that is, as it relates to obtaining homozygous or near-homozygous lines. The remainder of the discussion will consider this relationship.

ORR-EWING (1956), after careful investigation, concluded that loss of embryos following self-fertilization was probably due to lethal and detrimental recessive genes[6]) brought into the homozygous state through selfing. We will assume that lethal and detrimental genes are distributed randomly over the chromosomes.

If a tree carries a single recessive embryonic lethal or detrimental (Aa) and is selfed, 25 percent (1-3/4) of the selfed progeny will be of the lethal or detrimental genotype (aa) and 75 percent (AA, Aa, Aa) will not. If a tree carries two such genes and they are independent, about 56 percent $[1-(3/4)^2]$ of the selfed progeny will carry at least one lethal or detrimental allele in the homozygous state. Like-

---

[4]) Personal communication, 1967.

[5]) Author, unpublished results.

[6]) Detrimental recessive genes are those which lower family survival when homozygous, but to a lesser extent than lethal genes.

wise, if a tree carries n embryonic lethals or detrimentals and they are independent, $[1-(3/4)^n] \times 100$ percent of the selfed progeny will be homozygous for at least one of the n recessive alleles.

However, the lethal and detrimental genes will not all be independent. There is a certain probability that some of the lethal chromosomes or lethal chromosome segments will carry, linked together, two or more lethals or detrimentals at different loci. To account for this, and also for the cumulative effect of detrimental genes, the load of deleterious genes is calculated in terms of "lethal equivalents"[7] rather than lethals. Number of lethal equivalents per individual has been determined for the 35 tested Douglas-fir by equating number of lethal equivalents to $-4 \ln R$, where ln R is natural logarithm of the relative yield of filled self-pollinated seeds given in *Table 2* (Morton, Crow, and Muller, 1956).

It was stated earlier that relative selfed seed yield averaged 11.3 percent (mean) and 7.5 percent (median). From the above percentage it can be calculated that, on the average, coastal Douglas-fir carries an estimated 8.7 lethal equivalents per tree, and that the median tree carries an estimated 10.4 lethal equivalents.

In a selfing study in pine (Fowler, 1965), it was suggested that the number of lethals per tree might be higher because more than one fertilization occurs per ovule and more than one embryo can start development in each ovule. Polyembryony has also been observed in Douglas-fir (Orr-Ewing, 1956). However, it has been further observed by Orr-Ewing (1956, p. 31) that careful examination of the ovules throughout his investigation showed that "collapse was general (i. e., included all material within the seedcoat) whenever the terminal embryo had collapsed and that there was never any question of a second embryo taking its place."

The estimates of lethal equivalents given in this paper were based on one embryo per ovule. On this basis, they are minimum estimates. That is, if there are ovules where the fate of the terminal embryo does not determine the fate of the seed contents, then the estimate of lethal equivalents given above will be an underestimate.

However, another factor also needs to be considered, namely, that number of lethal equivalents appears to be overestimated except when determined at very low levels of inbreeding (Malogolowkin-Cohen *et al.*, 1964). This is probably because the deleterious effects of recessive detrimental genes tend to act synergistically when several such genes become homozygous at the same time in the same individual. In this study the trees were self-pollinated, so estimates were made when the inbreeding coefficient equaled 0.50, a moderately high level of inbreeding. Consequently, some overestimation may be expected from this source, particularly for trees carrying large numbers of detrimental genes. Considering, therefore, that there are plausible causes both for over and for underestimation of the number of lethal equivalents, it would appear that estimates based on one effective embryo per ovule are the most reasonable at the present time. Therefore, one embryo per ovule has been the basis for calculating lethal equivalents in this paper.

A distribution of trees according to number of embryonic lethal equivalents is given in *Table 4*. Attempts were made to fit this distribution to theoretical distributions, but the fits were poor. Consequently, the following implications to inbreeding are based on the observed distribution.

*Table 4.* — Frequency distribution of embryonic lethal equivalents in 35 coastal Douglas-fir trees.

| Number of lethal equivalents | Number of trees | Relative frequency |
|---|---|---|
| 0 | — | |
| 1 | — | |
| 2 | — | |
| 3 | 2 | 0.0572 |
| 4 | 1 | 0.0286 |
| 5 | 1 | 0.0286 |
| 6 | 3 | 0.0858 |
| 7 | 2 | 0.0572 |
| 8 | 4 | 0.1142 |
| 9 | 3 | 0.0858 |
| 10 | 6 | 0.1718 |
| 11 | 2 | 0.0572 |
| 12 | 3 | 0.0858 |
| 13 | 2 | 0.0572 |
| 14 | — | |
| 15 | 1 | 0.0286 |
| 16 | — | |
| 17 | 1 | 0.0286 |
| 18 | — | |
| 19 | 1 | 0.0286 |
| 20 | — | |
| 21 | — | |
| 22 | — | |
| 23 | 1 | 0.0286 |
| 24 | 1 | 0.0286 |
| 25 | — | |
| 26 | — | |
| 27 | 1 | 0.0286 |

*1. — Obtaining completely homozygous individuals:*

Even though average self-fertility is quite low in coastal Douglas-fir, it is not so low that it precludes tree improvement techniques including an initial phase of inbreeding (Orr-Ewing, 1957 a, 1965). On the other hand, there are reasons to expect that if an inbreeding phase is prolonged, or if complete homozygosis is sought, difficulties may be encountered.

In order to produce nearly, or completely, homozygous individuals from prolonged inbreeding or doubled haploids (Chase, 1952; Stettler, 1966), it will be necessary to produce or find lethal-free haploid sets of chromosomes. The distribution in *Table 4* indicates that such sets are rather rare in nature but that they can be found.

For example, in the distribution in *Table 4* no tree carries less than the equivalent of three lethals, about six percent carry three lethals, and about nine percent carry four or fewer lethals, etc. If an individual carries one chromosome with the equivalent of a recessive lethal, one-half of the haploid sets derived from meiosis in that individual would be lethal-free and could form the basis for a homozygous diploid; if two different chromosomes (not homologous) carry lethals, one-quarter of the haploid sets derived from meiosis would be lethal-free. In general, the proportion of lethal-free haploid sets derived from meiosis would be approximately $(0.5)^n$, where n is the number of non-homologous chromosomes carrying recessive lethals.

It can be seen that both the proportion of lethal-free haploid sets and yield of sound selfed seed go down as the number of lethals increases. By the time the number of

---

[7] A "lethal equivalent" can represent a fully lethal gene or the accumulation of detrimental genes whose effects are equal to one lethal.

lethals reaches three, the minimum observed in this study, the relative yield of sound selfed seed in down to about 47 percent of outcross yield, and only about 12 percent of the haploid sets derived from meiosis would be lethal-free. This means that to obtain doubled haploids both the technique for producing them must be efficient and an extensive search probably is necessary for individuals with high self-fertility.

2. — *Obtaining partially homozygous individuals:*

High incidence of embryonic lethals can, in other ways, make it more difficult to achieve high levels of homozygosity. For example, in the absence of recessive lethals, each generation of selfing should reduce the number of heterozygous loci by about 50 percent. In the presence of recessive lethals, the average heterozygosity of loci represented by or linked to recessive lethals will be reduced by only 33.3 percent rather than 50 percent because the homozygous recessive class will not live. Progress toward homozygosity will therefore be slowed, and average homozygosity of the $S_1$ families will differ depending upon the number of embryonic lethals carried by the parent.

If heterozygote advantage in fitness should exist, then selfing also will be less effective than expected in reducing heterozygosity. Further, an equilibrium frequency will be attained after many selfing generations, such that heterozygotes will always remain in the population (Hayman and Mather, 1953). It has not, and possibly cannot, be shown that embryonic lethals or chromosomal segments linked to them are overdominant for fitness in Douglas-fir. However, loci showing fitness overdominance when either homozygote is lethal have been experimentally observed in other outcrossing diploid organisms (Stern, Carson, Kinst, Novitski, and Uphoff, 1952), and tentatively postulated for seedling lethals in tree species (Squillace and Kraus, 1963; Williams and Brown, 1956).

Finally, it is also possible that reproductive fitness may be seriously reduced at relatively low levels of inbreeding. For example, in a recent report on a small population of Japanese quail with a genetic load estimated at 8.7 lethal equivalents, it was stated that there was a complete loss of reproductive fitness by the third generation of full-sib matings (Sittmann, Abplanalp, and Fraser, 1966). Because of its much greater reproductive capacity, Douglas-fir should tolerate higher levels of inbreeding, but still the indication is that many, perhaps most, lines may be lost after a few generations of selfing.

## Acknowledgements

The author wishes to express his gratitude to Drs. David Crumpacker and Bruce Wallace for helpful comments on the calculation of lethal equivalents.

## Summary

Ability of coastal Douglas-fir to set sound seeds following forced selfing was investigated by self- and cross-pollinating 35 trees at five locations in western Oregon. Self-fertility was expressed as a ratio of selfed to crossed yield of filled seeds.

Self-fertility was generally low, with the median tree yielding 7.5 percent as many seeds following self- as following cross-pollination. This indicates that in general there should not be much production of selfed seed under natural conditions even if natural self-pollination is found to be quite common. The major effect of low self-fertility will probably be a reduction in seed set.

Range of estimated self-fertilities, however, was large, extending from 0.1 to 45 percent. The occurrence of four trees with self-fertilities near or above 30 percent indicates that, from a few individuals, there is a considerable potential for natural selfing and production of selfed seedlings.

Genetic based postgermination seedling mortality due to traits such as albinism also contributed to inbreeding loss, reducing the number of germinated selfed seedlings by about 10 percent. There were no significant differences among the five locations in average self-fertility of sampled trees.

Minimum number of deleterious genes required to account for the observed seed loss following selfing was calculated. The median tree was found to carry the equivalent of about 10 lethal genes active during embryo development. This high load of deleterious genes may interfere with inbreeding programs whose goals are homozygous or near homozygous lines. Programs of the latter type not already begun should probably take advantage of the great variability in self-fertility, and start by searching for individuals of high self-fertility.

## Literature Cited

Allen, G. S.: Parthenocarpy, parthenogenesis, and self-sterility of Douglas-fir. J. Forest. **40**, 642—644 (1942). — Chase, S.: Monoploids in maize. *In:* Heterosis. Ames, Iowa. Iowa State Coll. Press. pp. 389—399. Ed. by J. W. Gowen (1952). — Duffield, J. W.: Techniques and possibilities for Douglas-fir breeding. J. Forest **48**, 41—45 (1950). — Fowler, D. P.: Effects of inbreeding in red pine, *Pinus resinosa* Ait. IV. Comparison with other northeastern *Pinus* species. Silvae Genetica **14**, 76—81 (1965). — Hayman, B. I., and Mather, K.: The progress of inbreeding when homozygotes are at a disadvantage. Heredity **7**, 165—183 (1953). — Istratova, O. T.: Flowering biology in *Pseudotsuga taxifolia.* Byulletin Glavnoj Botanicheskoj Sada (Moskva) **52**, 67—76 (1964). (In Russian). — Larsen, C. S.: The employment of species, types, and individuals in forestry. Roy. Vet. Agr. Coll. Yearbook, pp 69—222 (1937). — Malogolowkin-Cohen, Ch., Levene, H., Dobzhansky, N. P., and Solima Simmons, A.: Inbreeding and the mutational and balanced loads in natural populations of *Drosophila willistoni.* Genetics **50**: 1299—1311 (1964). — Morton, N. E., Crow, J. F., and Muller, H. J.: An estimate of the mutational damage in man from data on consanguineous marriages. Proc. Nat. Acad. Sci. (U.S.A.) **42**, 855—863 (1956). — Namkoong, G.: Inbreeding effects on estimation of genetic additive variance. Forest Sci. **12**, 8—13 (1966). — Orr-Ewing, A. L.: Inbreeding experiments with the Douglas-fir (*Pseudotsuga taxifolia,* [Poir.] Britton). Forest. Chron. **30**, 7—20 (1954). — Orr-Ewing, A. L.: An investigation into the effects of self-pollination on *Pseudotsuga menziesii* (Mirb.) Franco. Ph. D. thesis, Univ. Brit. Columbia (1956). — Orr-Ewing, A. L.: Further inbreeding studies with Douglas-fir. Forest Chron. **33**, 318—332 (1957 a). — Orr-Ewing, A. L.: A cytological study of the effects of self-pollination of *Pseudotsuga menziesii* (Mirb.) Franco. Silvae Genetica **6**, 179—185 (1957 b). — Orr-Ewing, A. L.: Possible occurrence of viable unfertilized seeds in Douglas-fir. Forest Sci. **3**, 243—248 (1957 c). — Orr-Ewing, A. L.: Inbreeding and single crossing in Douglas-fir. Forest Sci. **11**, 279—290 (1965). — Silen, R. R.: A laboratory seed separator. Forest Sci. **10**, 222—223 (1964). — Sittmann, K., Abplanalp, H., and Fraser, R. A.: Inbreeding depression in Japanese quail. Genetics **54**, 371—379 (1966). — Snedecor, G. W.: Statistical methods. The Iowa St. Univ. Press. Ames, Iowa. (1956). — Squillace, A. F., and Kraus, J. F.: The degree of natural selfing in slash pine as estimated from albino frequencies. Silvae Genetica **12**, 46—50 (1963). — Stern, C., Carson, G., Kinst, M., Novitski, E., and Uphoff, D.: The viability of heterozygotes for lethals. Genetics **37**, 413—449 (1952). — Stettler, R. F.: The potential role of haploid sporophytes in forest genetics research. Sixth World Forest. Congr. Madrid (1966). — Sziklai, O.: Variation and inheritance of some physiological and morphological traits in Douglas-fir. *In:* Joint Proc. Second Genet. Workshop Soc. Amer. Forest. & Seventh Lake States Forest Tree Impr. Conf. U.S. Forest Serv. Res. Paper NC-6, pp. 62—67 (1966). — Wheat, J. G.: The effect of self-pollination in Douglas-fir. IFA Tree Impr. Newsletter 2, 6. Ind. Forest. Ass. (1965). — Williams, W., and Brown, A. G.: Genetic response to selection in cultivated plants: gene frequencies in *Prunus avium.* Heredity **10**, 237—245 (1956).