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Reprinted from FOREST SCIENCE
Vol. 22, No. 2, June 1976
pp. 155-159

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Self-Pollination Effects on Seed and Seedling Traits in Noble Fir

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Abstract. Noble fir trees yielded on the average 69 percent as many sound seed after self- as after cross-pollination. Seed weight, germination percent, and seedling survival during 3 years were not affected by type of pollen. Selfed-offspring had significant inbreeding depression in 3-year height (24 percent). There was no indication that the generally low germination capacity of filled, healthy appearing seeds is due to natural self-pollination. ***Forest Sci. 22:155–159.***

Additional key words. Inbreeding, inbreeding depression, seed germination, seed weight, seedling survival.

ARTIFICIAL REGENERATION practices in noble fir (*Abies procera* Rehd.), an important upper slope conifer in western Oregon and Washington, are complicated by the relatively low germination percent characteristic of the species. This research tested the possibility that inbreeding could be the cause of low germination, and also compared the self fertility of noble fir with other northwestern conifers. Low germination capacity of fir seed in general has been attributed previously to inbreeding depression, as well as to such factors as fragility of seedcoat, embryo dormancy, and immaturity at the time of collection (Mergen and others 1964).

Materials and Methods

The study involved 10 noble fir trees growing in a stand at 1500-meter elevation in the Cascade mountains in central Oregon. The stand had scattered small openings and a large clearing upslope from the study area. One study tree was in the large clearing, the remainder on or near the edges of the small openings. Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) was a minor component of the stand.

Cross- and self-pollinations were made in 1968, a year of heavy seed production. Cross-pollen was an equal-volume mix from 8 to 10 other trees (exact number not recorded) separated by a minimum of 200 meters from seed trees. Self- (S), cross- (C), and wind-pollinated (W) cones were collected and processed by hand. Seeds were X-rayed and separated into "flat," "round empty," and "round filled" classes.

Fertility was determined for each pollen treatment by dividing number of filled seeds by number of round seeds (normal appearing empty and filled seeds). Relative self-fertility (RSF) was indexed by the ratio (Sorensen 1969),

$$\frac{\text{fertility after self-pollination}}{\text{fertility after cross-pollination}}.$$

Lethal equivalents were calculated from RSF's (Sorensen 1969) assuming one and two embryos per ovule (Koski 1973).

Seeds were soaked overnight in distilled water, moist stratified at 3°–4°C for 2 weeks, then germinated at 30°C day/20°C night temperatures with 12-hour photoperiods and thermoperiods. Germination per-

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TABLE 1. Relative self-fertility[1] and lethal equivalents[2] for noble fir trees.

Seed tree number	Filled seeds/round seeds after		RSF	Lethal equivalents assuming	
	Self-pollination	Cross-pollination		One embryo per ovule	Two embryos per ovule
38	0.57	0.48	1.18	0	0
1	.39	.36	1.07	0	0
36	.46	.59	.78	1.0	2.6
19	.41	.61	.68	1.6	3.4
18	.39	.62	.63	1.8	3.7
37	.44	.70	.63	1.8	3.7
26	.34	.54	.63	1.8	3.7
5	.26	.43	.59	2.1	4.1
6	.25	.58	.43	3.4	5.6
28	.06	.20	.29	5.0	7.4
Mean	.36	.51	.69	1.8	3.4

[1] Relative self-fertility =

$$\frac{\text{Filled seeds per cone after selfing/total seeds per cone after selfing}}{\text{Filled seeds per cone after crossing/total seeds per cone after crossing}}$$

[2] Lethal equivalent refers to a group of mutant genes, of unknown number, which, if combined, would cause on the average one death (Morton and others 1956). It includes the effect of deleterious as well as fully lethal recessive genes, and one lethal equivalent is approximately equal to the mortality from one fully lethal recessive gene with Mendelian segregation.

cent of filled seeds was calculated from two 100-seed replicates for each pollen type-seed tree combination.

Seedling growth and survival tests were started in the spring of 1970 with progenies from six parents. Progenies of the other four trees could not be included because of poor germination. Freshly germinated seeds were planted at 10-centimeter spacing in raised coldframes filled with agricultural loam.

Family sizes for seed traits were 100 to 200 seeds and for growth rates, 30 to 40 plants. One seed tree had fewer seedlings, 13 to 25 in each family. Survival, growth, and total height were tallied during three growing seasons.

Progenies were grown in family rows in three randomized complete blocks. For statistical analysis, blocks were ignored and seed trees served as replications. Orthogonal contrasts, S vs C and W vs (S + C) were made. The W vs (S + C) contrast was designed to test pollination bag effects and was limited to seed traits. To the extent that S- and C-pollination also affect these traits, bag effect and pollen type can be confounded. In the present test, seed weight and cotyledon number did not differ between S- and C-seeds. Thus, the confounding should be negligible. Arcsin transformations were used for the traits expressed in percentages.

Results

RSF's ranged from 0.29 to 1.18 (Table 1) and averaged 0.69. Calculated numbers of lethal equivalents on the zygote basis for the parent trees ranged from zero to five assuming one embryo per ovule and zero to seven assuming two embryos. From other coniferous species, there is evidence that the average number of effective embryos per ovule is closer to two (Koski 1973), but there is no information specifically on noble fir and other *Abies* species.

Pollen type (S-, C-, or W-) did not significantly affect round seed count per cone (average 378 round seeds per cone), seed weight (average 3.48 grams per 100 filled seeds), or germination percent. Germination was poor (average 11.5 percent) and erratic with no germination in some seed tree-pollen type combinations. A second test was conducted under different conditions—overnight soak in distilled water, 6-week stratification at 5°C, germination on sponge rock maintained at specific moisture content by weight, 8-hour photoperiods and thermoperiods, 30°C day/20°C night temperatures. Germination in this test was also poor and erratic. Germination speed was recorded but not analyzed because of small numbers of seeds which germinated.

Cotyledon number was significantly greater for S- than for C-families (Table 2), perhaps partly due to the slightly but nonsignificantly heavier S-seeds. An increase in cotyledon number accompanying inbreeding has also been reported in jack pine (*Pinus banksiana* Lamb.) (Fowler 1965) and Douglas-fir (Sorensen and Miles 1974). In Douglas-fir, C-seeds were heavier than S-seeds.

Inbreeding depression in growth was 20 percent after 1 year, 24 percent after 3 years. Nursery survival percentages did not differ among pollen types.

TABLE 2. Values for fertility, seed, and seedling traits which showed significant differences among cross- and self-pollination noble fir families. Values of wind-pollination families are included and tested where appropriate.

Item	Mean value for cross-pollination	Value relative to cross-pollination	
		Self[1]	Wind[2]
Filled seeds per cone (number)[3]	199.00	.68**	.87
Fertility (filled seeds/round seeds)	.515	.69**	.90
Cotyledon number	5.12	1.04**	1.03[n.s.]
First-year epicotyl length (cm)	2.98	.80**	.98
Second-year growth (cm)	10.6	.80**	1.01
Third-year growth (cm)	9.27	.66**	.91
Third-year total height (cm)	24.4	.76**	.97

[1] Significance level of the mean difference with cross-pollination; n.s.—nonsignificant; **—significant at the 1-percent level.

[2] Significance level of the mean difference for the contrast, wind- vs (self- plus cross-pollination), which tests the "bag effect." Where no superscript appears, the contrast was not considered appropriate.

[3] Units in parentheses apply only to column headed "Mean value for cross-pollination."

Discussion

No other reports on self-fertility in *Abies* species have been published. The RSF of 0.69 is high for conifers in general and in comparison with estimates for three other conifers of the western United States—0.11 for Douglas-fir (Sorensen 1971), 0.37 for ponderosa pine (*Pinus ponderosa* Laws.) (Sorensen 1970), and 0.53 for western white pine (*Pinus monticola* Dougl.) (Bingham and Squillace 1955).

Lack of differences among S-, C-, and W-progenies in nursery survival contrasts with reports on most conifers (Franklin 1970). A possible explanation is the lack of lethal mutants. Most of the excess mortality in nursery-raised Douglas-fir and ponderosa pine S-families was due to segregating lethals (Sorensen and Miles 1974). None of the noble fir S-families segregated for lethal effects.

Although there were only six S-families in the test and carriers of recessive mutants may have been missed by chance, the absence of aberrant types was unexpected. A larger sample of 156 nursery grown W-families (about 6,000 seedlings) also contained very few seedlings with detectable aberrant lethal phenotypes. Together, these observations suggest that post-embryonic lethals are uncommon in noble fir.

The coefficient of inbreeding in W-progenies from natural stands of conifers is reported to be quite low (Franklin 1971). Because there do not seem to be phenological and physiological barriers to S-pollination and fertilization, it has been assumed that abortion of inbred embryos is the primary mechanism by which production of inbred seedlings is restricted under natural conditions. The effectiveness of embryo abortion can be illustrated with an example based on data from Douglas-fir (Sorensen 1971, 1973) and noble fir (this paper). In Douglas-fir, controlled S-pollinations yield about 11 percent as many filled seeds as C-pollinations. Using this figure and, for purposes of illustration, a proportion of S-pollinations of 0.40, the frequency of S-seedlings in a W-progeny can be calculated with the formula:

$$\text{Frequency of S-seedlings} = \frac{RX}{(1-X)+RX},$$

where R = relative viability (RSF in Table 1) of S-embryos
X = proportion of S-pollinations.

Given $R = 0.11$ (from the Douglas-fir data) and $X = 0.40$, the frequency of S-seedlings in the W-progeny would be 0.068 or about 7 percent. (If multiple embryos were assumed, the result would be affected by competition between S- and C-embryos in the same ovule and by C-embryo mortality; however, for purposes of comparison here, only single embryos are considered.)

In noble fir with an RSF of 0.69, the situation would be quite different. A frequency of 40-percent S-pollination from the W-pollen cloud would result in about 32 percent S-seedlings in the W-progeny using the same assumptions, and if there were no mechanism other than embryo abortion which restricted the production of inbred seedlings.

Compared with other reports, the figure of 32 percent seems high. Unfortunately, it is not possible to confirm or reject it with our data. Performances of S-, W-, and C-families give ambiguous comparisons. For example, percentages of filled seeds (following Franklin 1971), cotyledon numbers, 1st-year epicotyl lengths, and 3d-year total heights indicated, respectively, that 37, 60, 10, and 12 percent of the W-seedlings were equivalent to S-seedlings. Cotyledon number is influenced by seed weight, but in this case the seed weights of the pollen types did not differ. The percentage based on filled seeds does not include the inbreeding effect on germination, but again germination percent did not differ between pollen types. Thus, there seems to be little basis for preferring one estimate over the others in this test.

Observations on flowering phenology and distribution of strobili in the crown are also not conclusive. Pollen shedding and female receptivity are synchronized (Franklin and Ritchie 1970). Male and female strobili are separated with the females toward the top. Other species with female strobili spread over the crown yield a lower frequency of S-seedlings in the upper than in the lower crown (Fowler 1965, Franklin 1971), presumably because of less S-pollination in the upper crown. Perhaps the spatial separation of the two flower types in noble fir is also effective in limiting S-pollination. In any case, it does raise the interesting question of how species with high self-fertility limit the production of self-seedlings under natural conditions.

Finally, the study does not indicate that selfing is in any way responsible for poor germination of commercial collections of noble fir seed. Although germination in general was poor to moderate, it did not differ among pollen types, nor was the production of round seeds per cone affected by pollen type.

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