Self-pollination Effects on Douglas-fir and Ponderosa Pine Seeds and Seedlings

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Inbreeding is an important factor in tree improvement. Avoidance of inbreeding is, in most tree species, one way of upgrading seed and plant quality. In previous articles (Sorensen, 1970, 1971), the effect of self-pollination on seed production in Douglas-fir (Pseudotsuga *menziesii* (Mirb.) Franco) and ponderosa pine (Pinus ponderosa Laws.) was reported. Development of the inbred families is described in the present paper. Cross- and wind-pollination progenies are included for comparison.

Estimates of inbreeding depression have been published for Douglas-fir (Orr-Ewing, 1954, 1957, 1965), but not, as far as we can determine, for ponderosa pine. Inbreeding depression in conifers in general has been surveyed by Franklin (1970).

Materials and Methods

Self-, cross-, and wind-pollination progenies (hereafter referred to as S, C, and W progenies) from 31 Douglas-fir and 17 ponderosa pine seed trees were included in the test.

Descriptions of seed trees, stands, pollination and seed handling methods were given in Sorensen (1970, 1971). Trees and seeds of the two species were treated similarly except that pine seeds were X-rayed and only seeds with fully developed embryos were used for seeding. The final separation of Douglas-fir seeds was in an airstream; there was no separation based on embryo development.

Nursery trials of Douglas-fir were started in 1964, 1965, 1966, and 1968. Germinated seeds were spotted at 7.6 cm X 7.6 cm spacing in nursery coldframes. Germinated ponderosa seeds were spotted at 7.6 cm X 7.6 cm spacing in 1969 and transplanted to 10.2 cm X 10.2 cm after the 1st year. (The purpose of the transplanting was to equalize the competitive effects to which the inbred plants were subjected. The ponderosa seedlings were bulkier and less tolerant to shade; therefore, a wider spacing seemed advisable for this species.) Douglas-fir nursery tests were terminated after the 2d-year measurements, ponderosa pine after 3d-year measurements.

Progenies were planted in family rows with the three types of families from each seed tree adjacent to one another. Progeny size depended on self-fertility and mortality, and averaged about 60 seeds (range 10 to 100) and 35 seedlings (range 5 to 75) for Douglas-fir and about 95 seeds (range 15 to 100) and 40 seedlings (range 25 to 45) for ponderosa pine.

Analyses were based on family means weighted for unequal seed and seedling numbers. The progeny of each tree with years confounded served as replications. Comparisons included the orthogonal contrasts of S vs. C progenies and W vs. *S* plus C progenies. The latter comparison was of little genetic interest but was used to test a suspected effect of conelet bagging on some seed traits.

Results and Discussion

Self- vs. *Cross-Pollination* Progenies

Douglas-fir sets about 10% as many seeds after self-pollination as after cross-pollination (Sorensen, 1971), and ponderosa pine, about 35% (Sorensen, 1970). That is, Douglas-fir has greater inbreeding depression in self-fertility. Nevertheless, in the traits listed in Table 1, and particularly those associated with growth and survival, the inbreeding depressions of the two species were quite similar. This indicates considerable independence of the genetic factors underlying embryonic mortality and those underlying seedling mortality and inbreeding depression in growth.

In Douglas-fir, seed weight was significantly less for *S* than for C progenies; there was no difference among the two family types in ponderosa pine. This might be due to the exclusion of seeds with underdeveloped embryos in the latter species.

Germination percentages in both species were less for filled seeds from S families than for filled seeds from C families. Sorensen (1970) found reduced germination of S seeds in ponderosa pine to be due primarily to higher percentage of seeds with weakly developed embryos than in the C families. We found what appeared to be corroborative evidence for this in X-rayed Douglas-fir seed, but Orr-Ewing (personal communication, 1972) reported no increase in frequency of poorly developed embryos in S seed, even in the S_2 generation.

Self-pollination had a different effect on cotyledon numbers in the two species. The difference between family types was not significant for ponderosa pine, either with or without adjustment for seed weight. However, in Douglas-fir, S seedlings had significantly higher number of cotyledons than C seedlings, i.e., the inbreeding effect was toward increased numbers. Fowler (1965) reported a similar effect in Pinus *banksiana.*

Comparative height growth of S and C families showed a 1st-year inbreeding depression of 18% in Douglas-fir and 21% in ponderosa pine, and an increase with age. An increase in percent inbreeding depression with time indicates that elongation (growth) rates of S families are less than those of C families.

The difference between family types in growth after the 1st year could arise from inherent differences in growth rate, from different positions on the sigmoidal growth curve (e.g., if the positioning on the X-axis of the curve is a function of size rather than time), or both. If growth curve position is the dominant factor, the increase in percent inbreeding depression with time should continue only until both family types are on the linear (or nearly linear) portion of the curve. If at this time the curves should become parallel, the percent inbreeding depression would even decrease with age.

Other reports are somewhat inconsistent on the relationship between inbreeding depression expressed as a per-

Table 1. — Values for seed and seedling traits from self-, cross-, and wind-pollination Douglas-fir and ponderosa pine families. (Mean values are given for the cross-pollination treatment; relative values are for self- and wind-pollination treatments.)

Trait	Species	Mean value for cross-pollination	Value of self- and wind- relative to cross-pollination[1]	
			Self[2]	Wind
Seed weight (gm/100 cleaned seeds)[3]	D-f.	1.439	0.93*	0.80***
	P.p.	4.615	1.03^{ns}	1.13***
Germination (percent)	D-f.	94.6[4]	0.96*	0.95^{ns}
	P.p.	94.4[4]	0.94^{nt}	0.99^{ns}
Germination speed (days to 50%)	D-f.	5.53	1.06^{ns}	1.00^{ns}
	P.p.	11.29	0.74^{ns}	0.46*
Cotyledon number (adjusted for seed weight)	D-f.	6.93	1.05***	1.04***
	P.p.	9.25	0.99^{ns}	0.93^{ns}
First-year growth (epicotyl length in cm)	D-f.	4.49	0.82***	0.89
	P.p.	2.70	0.79***	1.06
Second-year growth (cm)	D-f.	21.4	0.72***	0.93
	P.p.	3.86	0.74***	1.03
Third-year growth (cm)	P.p.	19.0	0.68***	0.99
First-year survival (percent)	D-f.	98.5[4]	0.89***	0.98
	P.p.	97.8[4]	0.91*	1.00
Survival of 1st-year seedlings to end of 2d year (percent)	D-f.	98.4[4]	0.92***	0.99
	P.p.	100.0[4]	0.97*	1.00
Survival of 2d-year seedlings to end of 3d year (percent)	P.p.	99.9[4]	0.99^{ns}	1.00

[1] Relative values = $\frac{\text{mean value for self- or wind-pollination treatment}}{\text{mean value for cross-pollination}}$

[2] Orthogonal contrasts, using mean values for all pollination treatments, included self- vs. cross-pollination and wind- vs. (self- + cross-pollination). The latter comparison was of interest only with regard to certain seed traits, as reported in the text. Separate tests, wind vs. self or wind vs. cross, were not appropriate. ns = nonsignificant; * = significant at 5% level; *** = significant at 0.1% level; nt = test not possible.

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

[4] Mean values for cross-pollination retransformed from arcsins.

centage and age. Snyder (1972) found inbreeding depression in slash pine (*Pinus elliottii* var. *elliottii* Engelm.) to increase from 21 to 34% from years 1 to 5; Gansel (1971) reported little change in inbreeding depression with age for the same species. Bingham and Squillace (1955) recorded inbreeding depressions of 11, 21, and 21% for 1st-, 2d-, and 3d-year nursery heights of western white pine (*Pinus monticola* Dougl.). Koski (1973) gave 1st-, 2d-, and 3d-year heights for 3 groups of S and W *Picea abies* (L) Karst. families. In 2 groups the inbreeding depression was the same after the 2d and 3d years. In the 3d group, which showed little 1st-year depression, there was still an increase in inbreeding depression between years 2 and 3.

Little change in percent inbreeding depression in height was observed in *Pinus sylvestris* between the ages of 6 and 16 years (Dengler, 1939, Scamoni, 1950) and in *Picea abies* between the 8th and 27th years (Langlet, 1940—41). The heights for *Picea abies* were recently reported up to age 61 (Eriksson, Schelander, and Åkebrand, 1973). These results showed the inbreeding depression to have dropped from 54 to 28% between years 27 and 61 (determined from Fig. 4 of Eriksson, Schelander, and Åkebrand after omitting the progeny of tree 5 which had only one inbred plant). S and W progenies were 4.6 and 9.9 meters tall at 27 years of age. They grew 10.1 and 10.4 meters, respectively, between the ages of 27 and 61. Since relative growth rate includes a term for starting size as well as for growth, it is evident that relative elongation was greater for S than for W progenies during this time span. Inbreeding depression in diameter growth followed the same pattern.

Site, differential competition effects, and differential mortality could cause ambiguities in test results. Nevertheless, the evidence seems to show that inbreeding depression is primarily associated with plant establishment and early development.

Barnes (1964) reported that growth depression was less in seedlings from completely self-fertile trees than in seedlings from partially self-fertile trees of *Pinus monticola*. Snyder (1968) noted a similar but weak relationship with many exceptions in *Pinus elliottii* var. *elliottii*. In the present material, percent inbreeding depression, in final nursery seedling heights, was found to have nonsignificant correlation with self-fertility (r = 0.316 with 17 d.f. for Douglas-fir; r = 0.123 with 15 d.f. for ponderosa pine). Lack of correlation within species would be consistent with the observation that the two species had similar inbreeding depressions, although their self-fertilities were different

Barnes (1964) also reported an increase in inbreeding depression with increasing altitude of the seed tree. We tested this relationship by regressing percent inbreeding depression in final nursery seedling heights on altitude of the seed parents. Nineteen pairs of S and C Douglas-fir families which were seeded in 1968 were used. Elevation of seed trees ranged from 50 to 1,350 meters and inbreeding depression from 50.3 to —11.2. The regression coefficient was not significant (b = 0.003).

Seedling survival was lower in S than in C families. Most of the increased mortality in the S families was due to deleterious major genes, such as those causing chlorophyll deficiencies.. When this mortality was excluded from the analyses, the differences in survival between S and C families were nonsignificant in both species with the exception of 2d-year survival in Douglas-fir. Mortality due to major genes, identified through recurring characteristic phenotypes in certain S families, was 9.4 and 9.7% in Douglas-fir and ponderosa pine, respectively. No similar mutant phenotypes were observed in any of the C families.

Wind-Pollination Families

Douglas-fir W seeds were, respectively, 14 and 20% lighter than S and C seeds. Conversely, ponderosa pine W seeds were 9 and 13% heavier than those from S and C pollination.[1] Heavier S than W seeds have been previously reported for Douglas-fir (Orr-Ewing, 1954, 1957). Heavier C than W seeds have been observed by Sorensen (1973 b and unpublished data).

Relative size of S, W, and C seedlings is sometimes used to provide some measure of the frequency of S seedlings in families produced under conditions of natural pollination. Because of the relationship between seed weight and seedling size, statistical adjustment for seed weight or pairing by weight classes is required (Squillace & Bingham, 1958). The "bagging effect" referred to above, when it occurs, complicates the adjustment. With reference to Douglas-fir, for example, seeds from S and C pollination were similar in size, but the seedlings produced from them were very different. W seeds were much lighter than either of the other two types, but W seedlings were intermediate.

A plotting of this general relationship shows that adjustment for seed weight does not properly measure its effect on growth. Further, pairing by seed weight is impractical in species of low self-fertility, such as Douglas-fir, because many of the S families do not have enough seed to pair with that from the lighter-seeded W families.

To avoid these difficulties, Conkle (personal communication, 1972) suggested using mortality due to major genes as an indicator of the frequency of S seedlings in the W population. Mortalities which could be identified with major genes were 9.4, 0.6, and 0.0 (Douglas-fir S, W, and C seedlings) and 9.7, 1.1, and 0.0 (ponderosa pine S, W, and C seedlings). Percent S seedlings (or equivalent) in W population was calculated from ratio of mortality in W population to mortality in S population times 100. This calculation gave 6.4 and 11.3% S seedlings in the Douglas-fir and ponderosa pine W populations, respectively. The estimate for Douglas-fir is based on a portion of the same trees used in an earlier estimate (Sorensen, 1973 a). That estimate, based on more parents and much larger families, was 7.0%. Thus, this technique would appear to be suitable for estimating natural inbreeding.

The question of roguing inbred seedlings in the nursery was discussed by Snyder (1968). He reported that seedling size distribution of S and C seedlings overlapped too much for roguing to be effective. Barnes (1964), on the other hand, recommended nursery culling on the basis of slow height growth of S seedlings. The potential of nursery culling in Douglas-fir and ponderosa pine was evaluated by creating artificial populations from arrays of S and C seedlings from the same seed trees. The Douglas-fir population was based on families from 12 low-elevation seed trees, the ponderosa pine population on families from 16 medium-elevation seed trees. Two artificial populations were created, one with composition 95% C seedlings, 5% S seedlings, and the other with composition 90 and 10%, respectively. The results of culling nursery populations of 2-year-old Douglas-fir and 3-year old ponderosa pine seedlings were approximately the same for both species and both original population compositions. The average effect of 10, 20, 30, and 40% culling was to remove 38, 62, 71, and 79%, respectively, of the S seedlings and approximately 8, 16, 25, and 35% of the C seedlings from the original population.

Because of the overlap in S and C seedling sizes, culling of 10—20% of a nursery population would appear to be most effective in removing inherently slow-growing S seedlings and to contribute most to an upgrading of the growth potential of the planting stock. C seedlings are eliminated almost as rapidly as S seedlings at culling levels above about 20%.

Summary

Seedlings from self- and cross-pollination of Douglas-fir and ponderosa pine seed trees were compared in seed and nursery traits. Seed weight, germination percent, and germination speed were little to unaffected by selfing. Cotyledon number was increased in Douglas-fir and unaffected in ponderosa pine by selfing. Inbreeding depression was 18 and 21% in 1st-year seedling height for Douglas-fir and ponderosa pine, respectively, and increased with age. It was 11 and 9% in 1st-year survival, with most of the mortality associated with inbreeding ascribed to major gene effects. There was no relationship between inbreeding depression in height and self fertility, or between the former and elevation of the seed trees.

Performance of wind-pollination seedlings was included for comparison. The environment within the pollen isolation bags influenced seed weight and seedling size, increasing both in Douglas-fir and decreasing them in ponderosa pine. Frequency of self-pollination seedlings in the wind-pollination population was estimated at 6.4% (Douglas-fir) and 11.3% (ponderosa pine) using percent mortality due to major gene effects. An estimate could not be made from growth data because of seed weight effects. Using artificial populations of self- and cross-pollination seedlings, the effect of nursery culling on removal of self-pollination seedlings was described.

Key words: Douglas-fir, ponderosa pine, inbreeding, depression, nursery culling.

Zusammenfassung

Sämlinge, die aus Selbst- und aus Fremdbestäubung sowohl bei Douglasie wie auch bei der Ponderosa-Kiefer hervorgegangen waren, wurden verglichen. Samengewicht, Keimprozent und Keimdauer waren durch die Selbstung nur wenig bis nicht beeinflußt worden. Die Cotyledonenzahl stieg bei der Douglasie und bei den nicht beeinflußten Selbstungskeimlingen der Ponderosa an. Die Sämlingshöhe im 1. Jahr zeigte bei der Douglasie 18% und bei der Ponderosa 21% Inzuchtdepression, die mit zunehmendem Alter weiter anstieg. Es überlebten im 1. Jahr 11% bzw. 9% der Sämlinge, wobei ein Großteil der Mortalität der Wirkung von Major-Genen zugeschrieben wird. Es bestand fer-

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[1] C. W. Wang (personal communication, 1973) points out that ponderosa pine seed size may be influenced by seed number per cone and that cone size may be affected by inadequate pollination. Therefore, it should be noted for this test that there were more seeds per cone after wind- than after cross-pollination and that over 65% of the seeds were filled in both cases. However, the influence of seed number per cone may have been responsible for self-pollinated seeds being slightly heavier than cross-pollinated seeds.

ner keine Beziehung zwischen der Inzuchtdepression in der Höhe und der Selbstfertilität der Elternbäume oder ersterer und der Höhenlage der Herkunft dieser Bäume.

Das Verhalten von Sämlingen aus Windbestäubung wurde in den Vergleich einbezogen. Dabei zeigte sich, daß die Umwelt in den Isolierungstüten das Samengewicht und die Sämlingsgröße beeinflußt hatte. Beides stieg bei der Douglasie an und nahm bei der Ponderosa ab. Die Häufigkeit von Selbstungssämlingen in einer frei abgeblühten Population wird bei Douglasie auf 6,4% und bei der Ponderosa auf 11,3% geschätzt. Dies ergibt sich, wenn das Sterblichkeitsprozent, das durch Majorgen-Effekte hervorgerufen wird, zugrunde gelegt wird. Eine Schätzung bei Wuchsdaten konnte wegen der Auswirkungen der Samengewichte nicht durchgeführt werden. Bei künstlichen Populationen aus Selbstungs- und Fremdbestäubungs-Sämlingen wurde der Effekt von Baumschulauslese auf die Entfernung von Selbstungssämlingen beschrieben.

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The Inheritance of Heartwood Formation in Pinus radiata D. Don.

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Introduction

The modifications to wood anatomy associated with heartwood formation are important in many aspects of utilisation, both for sawn timber and pulp and paper. These changes result in a greatly increased resistance to the movement of liquids from one cell to another so that seasoning difficulties are increased and the material is less readily penetrated by preservatives and pulping liquors. In addition, there is evidence that the heartwood of coniferous trees almost invariable contains more resin than the sapwood, and the difference is particularly marked in the pines (Mutton 1962; Keith 1969). The presence of resins can also influence the usefulness of the raw material, such as for example by adversely affecting the paper-making qualities.

Heartwood development begins some distance above ground level in pines (Liese 1936; Trendelenburg 1939; Paul 1952), and then extends both towards the crown and the butt. The age of its inception in *P. radiata* was noted by Harris (1954) as approximately 14 years from time of planting and the average rate of transformation of sapwood to heartwood was reported by Nicholls and Dadswell (1965) as approximately 0.5 growth rings per year. There is little published information on the inheritance of heartwood formation and the two available estimates of heritability were derived from *P. radiata* clonal material (Nicholls 1965 a). As it is not practical to investigate the variation in age at which heartwood begins to form, these estimates were based on the area proportion of heartwood.

There is a need to extend these preliminary estimates by work on both clonal and sexually reproduced material. Results of some further studies are reported below.

Materials

The trees for examination originated at three different localities.

Group 1. Material previously sampled in 1960 to obtain heritability estimates for the proportion of heartwood by Nicholls (1965 a) and growing in a *P. radiata* clonal trial established in 1939 at a spacing of 2.4 m × 2.4 m at Blue Range plantation in the Australian Capital Territory. The plantation is 25 km west of Canberra at an elevation of 780 m. The site slopes gently with an eastern aspect and soil is derived from Ordovician sediment. Mean annual rainfall is 1015 mm. Thirteen clones were sampled by selecting the first, middle and last trees of the row com-

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