

AN ABSTRACT OF THE THESIS OF

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Upon Seed Yields in a Douglas-fir Seedling Seed Orchard

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Effects of four inbreeding levels ($F = 0, 0.125, 0.25, 0.5$) and two supplemental mass pollination (SMP) methods (agitation of naturally shedding pollen with "air blaster" and pollen application from large "pollen wand" atomizer) were evaluated on seed of trees from eleven full-sibling Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) families in a 16-year-old seed orchard. SMP responses were tested at three crown levels on trees of three periods of

reproductive maturity. Cones covered during SMP treatments were used as controls.

Inbreeding did not significantly affect flat and round seed percentages or seed weights, but percent round, normal appearing seeds that were empty (PE) had a positive, linear relation to F. Filled seed yields after inbreeding did not differ among full-sib families; but variation among trees within families, as well as interactions between tree yields and inbreeding levels, were highly significant. At $F = 0.5$, PE was greater for seed orchard trees (98.2) than has been reported for trees in natural stands (90.9); juvenility and off-site planting of orchard trees may contribute to this difference.

Between $F = 0$ and 0.5, an increase in F of 0.1 was associated with an increase of 12% PE. Self-pollination appears to have its greatest impact on orchard seedset. Half- and full-sib matings also give reduced seed yields (relative to outcrossing), but the relatively high levels of inbred seed production should have their greatest impacts upon the productivities of the resulting seedlings. This contrast is discussed using inbreeding depression values from the

literature.

In contrast with other reports, air blast SMP did not increase seedset, but increased PE relative to the controls. Dense crowns of seedling seed orchard trees appear to intercept more self pollen than do the more open crowns of previously studied clonal orchards. Wand application of pollen, which was used only in conjunction with the air blast treatment, reduced PE to the level of the controls. The effect of crown levels (upper, middle, lower), as well as an interaction between crown levels and the phenology and SMP treatment applied to specific trees, were highly significant; causes of these results are speculated.

Practical implications are twofold. Because inbred seed production is high at $F = 0.25$ and 0.125 , seedling orchard designs must minimize the possibility of within-family mating. Secondly, crown form should be considered prior to use of some supplemental mass pollination treatments. In general, the dense crowns of young seedling orchard trees do not appear suitable for air blast SMP.

The Effects of Inbreeding and Supplemental Mass Pollination
Upon Seed Yields in a Douglas-fir Seedling Seed Orchard

by

Daniel W. Cress

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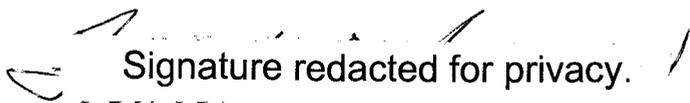
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THE EFFECTS OF INBREEDING AND SUPPLEMENTAL MASS POLLINATION UPON SEED YIELDS IN A DOUGLAS-FIR SEEDLING SEED ORCHARD

INTRODUCTION

Because increased yields and shortened rotations make tree improvement an attractive investment, genetic selection for improved growth rate of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco.) has been ongoing in the Pacific Northwest since the 1960's (Silen and Wheat 1979). Subsequently, seed orchards were established with the intent of generating tree seed of a desired genetic constitution. Orchards contain material reproduced from numerous selected trees; this material may be generated either asexually (clonal orchards), or sexually (seedling orchards). There are presently 1,911 acres (773 ha) of first generation Douglas-fir seed orchards in the Pacific Northwest, nearly 20% of which (378 ac., 153 ha) were established from seedlings (Bordelon et al. 1987).

Sexual recombination is typically utilized for creating advanced generation material; consequently, an even higher proportion of seedling seed orchards is expected in the future. Whether clonal or seedling, related individuals are typically interspersed among other

non-related orchard trees. Numerous computer programs have been developed for achieving orchard randomization (Marsh 1984) and are usually based on assumptions of random mating (panmixis) within orchard "neighborhoods", negligible gene flow from non-orchard trees, and some "acceptable" level of separation between orchard relatives.

Panmictic assumptions may not be valid. Natural self-pollination has been reported for Douglas-fir in both natural stands (Sorensen 1971) and in seed orchards (Erickson 1987, Omi 1983, Shaw and Allard 1982). The likelihood of mating between any two orchard trees is likely to be inversely proportional to the distance between them (Sorensen 1972) as well as being a function of reproductive phenology, fecundity, wind direction, pollen deposition rates, and competition from other pollen sources (Erickson 1987, Ho 1980, Owens and Simpson 1982, Webber 1987, Webber and Yeh 1987). A tree's maximum pollen shed and female receptivity will typically last for a period of from 4 to 9 days (Allen and Owens 1972, Barner and Christiansen 1962, Owens 1973, Owens et al. 1981) yet as many as 29 days have been reported between dates of maximum flowering on trees within the same orchard (El-Kassaby et

al. 1984). Mating can not occur between such extremely early and late flowering trees. Because reproductive phenology is under a high degree of genetic control, there is typically a continuum of sub-populations throughout an orchard's pollination season. Effective population sizes may often be much smaller than actual population sizes (El-Kassaby et al. 1984, El-Kassaby et al. 1986, El-Kassaby et al. 1988). Differential fecundity also may influence inbreeding frequencies (Erickson 1987, Koski and Muona 1986), and may be most pronounced in young orchards.

Inbreeding in most conifers is known to decrease seedset and produce seedlings of low growth rate, increased levels of defect, and less ability to withstand environmental stress (Bishir and Namkoong 1987, Bramlet and Bridgewater 1986, Franklin 1970, Griffin and Lindgren 1985, Omi 1983, Omi and Adams 1986, Orr-Ewing 1954, Orr-Ewing 1957, Piesch and Stettler 1971, Plym Forshell 1953, Plym Forshell 1974, Rehfeldt 1977, Shaw and Allard 1982, Sorensen 1970, Sorensen 1971, Sorensen et al. 1976, Sorensen and Miles 1982, Squillace and Kraus 1962). Avoiding the deleterious effects of inbreeding is important to tree improvement programs.

The degree of inbreeding is measured by the inbreeding coef-

ficient (F) and is defined as the probability that two alleles at a locus are identical by descent (Falconer 1981). Seedling orchards typically contain numerous trees of common ancestry (siblings) and have the potential for several levels of inbreeding. Because early orchards were primarily clonal, the effects of sib-matings ($F=0.125$ and $F=0.25$) have not been reported for Douglas-fir. Backcrosses to a common parent ($F=0.25$), full-sib matings ($F=0.25$), and half-sib matings ($F=0.125$) are also possible in seedling orchards, as are non-inbred ($F=0.0$) outcross pollinations (additional inbreeding levels are possible in later generations). The first objective of this study was therefore to investigate the influence of four inbreeding levels ($F= 0.0, 0.125, 0.25, 0.5$) upon seed production variables in a seedling orchard consisting primarily of full-sib families.

Seed orchard assumptions of negligible inflight of non-orchard (background) pollen also may be invalid (El-Kassaby and Ritland 1986, Silen 1962, Silen and Keane 1969). Appreciable pollen deposition has been reported at distances of over 60 km from the nearest timber stand, yet distances greater than 2 km between Douglas-fir trees are rare in the Pacific Northwest (Silen 1962).

Supplemental mass pollination (SMP) may be used in an attempt

to counteract problems associated with gene flow from non-orchard sources, inadequate pollen availability, and inbred matings. Numerous SMP techniques are operationally practiced in the Pacific Northwest, although the effectiveness of such programs has been variable (Brown 1987, Daniels 1978, El-Kassaby and Ritland 1986, Webber 1987, Wheeler and Jech 1985). SMP techniques and orchard conditions (spacing, age, crown form, etc.) may all affect treatment response. Therefore, the second objective of this study was to determine the effectiveness of two SMP methods in a seedling orchard and to contrast these results with those previously reported for clonal orchards (particularly as related to inbreeding, crown level, and reproductive phenology).

MATERIALS AND METHODS

SEED ORCHARD DESCRIPTION

The study was conducted in the Vernonia-B block of the J.E. Schroeder Seed Orchard located approximately 15 miles north of Salem, Oregon (Figure 1). This 400 acre complex is managed by the Oregon State Department of Forestry and contains 11 orchard blocks which are cooperatively owned by both public and private land-owners (Figure 2). Orchard blocks represent three coniferous species and nine breeding zones in western Oregon.

The 4.7 ha Vernonia-B block contains the oldest trees in the orchard complex and was established with two-year-old Douglas-fir seedlings in 1974. Although this block also contains several grafted clones, it was primarily established from seedlings of controlled two-parent crosses. The parent trees of these seedlings came from low elevation (250-500 m) sites in the northwest corner of Oregon.

Vernonia-B block was originally planted at regular 4' x12' (1.2 x 3.7 m) spacing; subsequent thinning, roguing, and mortality have resulted in extremely irregular spacing (Figure 3). Adjacent trees are now separated by as little as 12' (3.7 m) and as much as 332'

(101.2 m). In 1987 the remaining 658 trees had a mean height of 12 m and an average spacing of 8.4 x 8.4 m (71.3 m²/tree). Sixty parent trees were single-pair mated to generate the 31 full-sib families remaining in this block. Two pairs of families have common pollen parents and therefore are related as half-sibs. The 31 families contain an average of 19.2 trees each (range = 4 to 34).

The block was originally laid-out in a maplike fashion which mimicked the geographic arrangement of parent trees within the original breeding zone (Silen and Wanek 1986). The purpose of this design was to promote mating between orchard families of common geographic origin. Such a design may be beneficial from this standpoint, but it created a greatly increased potential for sib matings, particularly after orchard roguing (Figure 3). Full-sib and half-sib relatives are presently spaced as little as 12' (3.7 m) apart.

The Schroeder orchard is primarily surrounded by agricultural fields. A small mixed-conifer stand lies directly adjacent to the northeast corner of the orchard complex, but previous phenology records and prevailing wind directions indicated that this stand should be only a minor source of pollen contamination. The Vernonia-B block is separated from the similarly aged Vernonia-A

and Vernonia-C blocks by narrow access roads (Figure 2). All three blocks represent a common origin and are separated only because of differing ownerships. Because most other orchard blocks contained relatively young trees (at the time of this study [1987], range = 1 to 9 years from seed), between-block pollen contamination also should be negligible at the present time. Past years' data from several pollen deposition monitors have indicated that pollen contamination from other orchard blocks, or from outside sources, was negligible during the time of female receptivity. This conclusion also appeared to be supported by prevailing wind patterns, remoteness from upwind Douglas-fir stands, and the late flowering of the Vernonia blocks compared with native Willamette Valley trees (personal communication, W. Cook, Oregon State Department of Forestry).

Two methods of supplemental mass pollination (SMP) are operationally used in in this orchard. The intent of these treatments is to increase both the quantity and quality of pollen available to orchard trees. The first method involves pulling a "Turbo-mist" air blaster between orchard rows. This tractor-drawn high-velocity fan causes large volumes of air to be blown in an upward and inward direction relative to tree crowns with the intent of improving the orchard

pollen cloud through increased mixing of pollen from various trees. Fan velocity is maintained at a level which affects air movement for a distance of approximately 20 m vertically and 12 m horizontally. Depending upon weather conditions, this procedure is repeated approximately every other day throughout the pollination season. Due to the speed and low cost of this technique (\$12/ha/year, \$5/ac./year), it is used operationally throughout the entire orchard complex (personal communication, W. Cook, Oregon State Department of Forestry).

The second supplemental pollination method involves collecting mature catkins from selected trees and placing them in forced-air driers (approximately 2-3 km/hr air movement) in a heated and dehumidified room (approx. 30°C and 30% RH) for a period of 24-48 hours. The catkins are then lightly vibrated, the released pollen is sifted through 60 mesh screens, placed in airtight vials, and refrigerated at approximately 2°C. Pollen vials are later attached to a compressed air atomizer (hereafter called "pollen wand"), which is used to direct a spray of pollen onto female flowers of selected trees. Because of the large amount of time and labor involved with this SMP method, it is an extremely expensive procedure (\$620/ha/

year, \$250/ac./year). Operational use of the pollen wand therefore is restricted to only those orchard blocks with insufficient pollen production and seed yields, and to only those trees which flower early or late with respect to the remaining trees within these blocks (personal communication, M. Bordelon, Oregon State Department of Forestry). However, for the purpose of this study, the pollen wand treatment was used throughout the pollination season on trees within the Vernonia-B block. Normally, they would have received only "air blast" SMP .

STUDY TREE SELECTION

Study families were intended to sample the range in both timing of female receptivity (early/mid/late season flowering) and past yields of filled seeds (low/medium/high yield). Seed orchard records from two previous flowering years (1983 and 1984) showed that certain families reached maximum flowering earlier in the season than did others, but these relationships were not always consistent between years (Table 1). Seed yield records from 1985 and 1986 (in terms of pounds of viable seed per bushel of cones) showed marked variation between years and between families in any

given year (Table 2). Although orchard phenology and seed yield records did not represent the year in which the study was conducted, they were still used as guidelines for selection of study families. Delineation of family phenology and seed yield patterns was made by calculating average annual z-scores of these data (i.e. "late" flowering families were those with phenology z-scores ≥ 0.68) (Tables 1, 2, and 3).

Of the trees that had an adequate number of female flowers (≈ 200 well-distributed flowers per tree), four trees were randomly chosen from each of the selected families. Two of the four trees were used to assess the effectiveness of the Schroeder Orchard's supplemental mass pollination program, the other two were used to determine the effects of inbreeding upon seedset. Because 1987 was only a moderate year for cone and pollen production in this orchard block, desired phenology and seed yield standards could not always be met (Table 3).

STUDY TREE PREPARATION

Controlled pollination was used to assess the effects of inbreeding. Four levels of inbreeding were possible, but of the twenty trees

(representing 11 families) that were included in this portion of the study, pollen sources for half-sib matings ($F = 0.125$) were available for only three trees (representing 2 families). Pollen for self pollination ($F = 0.5$), full-sib pollination ($F = 0.25$), and outcrossing ($F = 0.0$) was available for all twenty selected mother trees.

Study tree branches which bore numerous female strobili were selected prior to flushing of female buds. Five cone samples were intended for later dissection and seed analyses. To compensate for potential cone mortality and to provide seed for other tests, up to 35 strobili were prepared per pollination treatment per tree (average = 21.2). Each selected branch was padded with cotton, male strobili were removed, and female buds were isolated with color-coded "Pollen Tector" isolation bags. Male strobili were allowed to remain on branches which were to be self-pollinated.

In total, 63 crosses were made (Table 4). The mating design for a hypothetical tree from family "(AxB)" is as follows: self = (AxB) x (AxB), full-sib = (AxB) x (AxB)', half-sib = (AxB) x (AxC), outcross = (AxB) x [polymix of at least 6 unrelated pollen parents: (CxD) + (ExF) + (GxH), etc.].

CONTROLLED POLLINATION TREATMENTS

Phenological development of female strobili was monitored on the study trees a minimum of two times per week. Observations were made on strobili actually enclosed within the pollination bags, because there is a tendency for these bags to hasten the maturation rate of the enclosed flowers (Sorensen and Campbell 1985). This same "greenhouse effect" was used on other trees in order to "force" the maturation of pollen sources which otherwise would not have been mature by the time needed for use.

Pollen collections were made at the time when pollen sacs of the microstrobili were almost fully expanded, but not yet heavily shedding. Catkin-bearing branch ends were cut off with pruning shears and loosely packed into labelled kraft paper bags. These bags were then transported to the PNW Forest and Range Experiment Station in Corvallis, Oregon, for further processing.

Detached branch ends were left sealed in the collection bags so that catkins matured intact on their branch tips. Bags were placed in a closed room which was maintained at a constant temperature of approximately 30°C. A portable dehumidifier was used to maintain a low relative humidity in the room. After drying for two days, bags

were individually removed from the room, shaken lightly, and the released pollen sifted through 60-mesh wire screens. Pollen was then poured into labelled glass vials and stored at approximately 2°C until needed.

Most trees were pollinated twice, some three times. Time of bud burst varies among strobili on the same crown and even within the same isolation bag. Each flower was first pollinated at the point of maximum receptivity (stage "B+1", according to Owens et al. 1981), and was re-pollinated approximately three days following the first application. The intent was to pollinate all strobili at or near the time of maximum receptivity.

Control pollination of each tree was made one treatment at a time (i.e. all self pollinations were finished before starting full-sib matings). Pollination involved briefly removing the isolation bag and carefully pouring pollen from the appropriate vial onto each individual female strobilus. Branches were tapped lightly in order to sift pollen between the flower bracts and to remove excess pollen which could potentially "clog" between the bracts and bud scales. Isolation bags were replaced immediately following pollination. It was felt that this technique provided a more uniform application of

pollen than would the alternative use of a pollen syringe (injecting pollen without removal of the isolation bag).

Great care was taken in making the crosses. Thus, it is assumed that all female strobili were pollinated with the intended pollen sources. Four factors, however, may have contributed to violations of this assumption.

First, there is the possibility of wind-borne pollen contamination during the time that isolation bags were removed for pollen applications. By working quickly and cautiously, and by pouring large quantities of pollen onto each flower, the likelihood of contamination should have been negligible. Results from self pollination (see below) show that such contamination from wind-borne pollen was at most, a rare occurrence.

A second source of error may have come from applying the wrong pollen in a particular treatment. A color coding system was used to avoid this problem (outcrossing pollen was in vials marked with blue tape and was applied to bags marked in the same color, selfs were marked with red, etc.), yet two errors are known to have been made. Although all pollinations of one color were to be completed before proceeding to a second color on a tree, full-sib pollen (orange vials)

was inadvertently applied to two red bags (intended for self-pollination). Although this mistake was caught and wrongly pollinated flowers destroyed, it did show that mistakes were possible, even with the color-coding system and careful work. In order to prevent mixing treatments between differing trees (e.g. applying pollen of red vials for tree "A" to red bags of tree "B"), each person carried pollen for only the tree being pollinated at that time.

A third error source could have resulted from differential pollen viability. Systematic testing of pollen samples with a 0.15% H_2O_2 solution (Schopmeyer 1974) indicated that all pollen lots had very high viabilities (95 percent or greater frequency of viable pollen grains), although this test is not totally reliable (Webber 1987). By applying large amounts of pollen to each female flower, it is quite unlikely that any ovule would have received only non-viable pollen grains.

Lastly, pollinating female strobili at a time after the point of maximum receptivity may bias seedset values by confounding the affect of inbreeding depression with that of pollen availability (Allen and Owens 1972). Although an attempt was made to pollinate each flower at the optimal time, there were occasionally flowers

which matured faster than anticipated. Late pollinations were noted in pollination records and such cones were omitted from seedset calculations, but it is possible that late pollinated (or unpollinated) cones may have been included in the five-cone samples that were later dissected.

To avoid potential insect damage to cones, isolation bags were allowed to remain on all branches throughout cone development. In order to identify any branch which might have lost its labelled bag, treated branches were also labelled with waterproof laundry tags. Any bags which deteriorated after the period of most insect damage were not replaced.

SUPPLEMENTAL POLLINATION (SMP) TREATMENTS

Eighteen trees were used to evaluate the effectiveness of the supplemental pollination treatments. Selected trees were full-sib relatives of those used in the inbreeding study and consisted of two trees from each of nine families (Table 3).

A sample of branches (bearing approximately twenty female cones per study tree) were prepared for bagging in the same manner as in the inbreeding study, but were not covered with isolation bags

at that time. In order that these flowers later could be located and bagged for isolation during the supplemental pollination treatments, prepared branches were marked with brightly colored surveyor's flagging and waterproof laundry tags. Prepared branches were distributed throughout tree crowns and were allocated in proportion to each tree's overall distribution of cones (thereby being concentrated towards the upper-crown).

Just prior to SMP treatment (pollen wand atomizer and/or turbo-mist air blaster), labelled branches were covered with the same type of "Pollen-Tector" isolation bags that were used in the inbreeding study. Operational SMP treatments were then applied to the remaining portions of these trees. Isolation bags were removed following dispersal of the resulting pollen clouds. Cones on these bagged branches were considered wind-pollinated controls. The same branches were re-isolated each time SMP treatments were applied. Care was taken in order to avoid damage during extensive handling of the repeatedly isolated branches.

Treatment followed normal operational practice; and therefore was restricted to days when winds were calm, foliage was dry, and female strobili were receptive. The air blast treatment was applied

to all orchard trees on ten occasions throughout the pollination season, while the pollen wand treatment was applied to the SMP study trees a total of six times.

CONE COLLECTION AND PROCESSING

All control-pollinated cones and the wind-pollinated controls from the SMP trees were collected from the study trees just prior to seedshed in August. A sample of 15 open-pollinated cones was also collected from the top, the middle, and the lower thirds of the cone-bearing regions of each study tree. Open-pollinated cones from the inbreeding study trees had received wind pollination and the air blast SMP treatments. The un-bagged cones from the SMP study trees had received wind pollination, air blast treatments, and the pollen wand treatments.

Cone abortion and frost damage reduced the number of cones available for harvest (Table 5). Where possible, five random but undamaged cones were selected from each tree-treatment combination. These cones were individually sealed in small kraft paper bags and hung in an open air shed for drying. Once ovuliferous scales began to flare, cones were moved to a dehumidified room, where

they were held at 20-25°C until processed.

A total of 835 cones were dissected by hand, and the total number of under-developed "flat seeds" and normal appearing "round seeds" was determined for each cone. The relative proportion of flat seeds (number flat seeds/[total seeds]) was determined for each sampled cone. Round seeds were classified from x-ray photographs as being either "filled" or "empty", and empty seed proportions (number round and empty seeds /number round seeds) were calculated. The terms "PF" and "percent flat seeds per cone" will be used interchangeably, as will the terms "PE" and "percent empty seeds per cone". These terms are defined as the associated proportions multiplied by 100.

Seeds damaged by insects were tallied as such, but were not included in calculations of percent empty seed. This approach was taken because damage was primarily due to "Douglas-fir Cone Gall Midge" (*Contarinia oregonensis*), an insect species which feeds on cone scales rather than upon the developing seed (Ruth 1980). Damage to these seeds probably occurred prior to the time of ovule fertilization. Of the 4,608 filled seed produced by control-pollinations, only 5 (0.11% of total) contained insect larvae; isolation bags

apparently protected the enclosed strobili from most seed insects.

Seed weights were measured to the nearest 0.001 gram. Filled seed weight was determined for each cone, as was that of a sample of each cone's empty seeds.

DATA ANALYSES

Regression analyses and least squares analyses of variance were carried out for the variables, total seed per cone (seed potential), PF, PE, and both filled and empty seed weights. Similar analyses were made using arcsine transformations of all proportions (Neter and Wasserman 1974, Section 15.5). Because transformations increased F-ratios and caused variances to be independent of mean values, analyses of untransformed data are not reported.

The experimental design of the inbreeding study can be best described as a modified split-plot with hierarchical classifications (personal communication, K. Rowe, Dept. of Statistics, Oregon State University; Petersen 1985, Section 10.2). This design was the result of trees being nested within families, and inbreeding levels being consistent for all treatments. Families, trees, and cones within treatments, were considered to be random effects; treatments

(inbreeding levels) were considered fixed (Table 6).

The extremely unbalanced data set complicated the analyses (Schultz 1955). In an effort to increase statistical sensitivity, analyses were conducted on both the entire data set (the "full model") and on a reduced set which did not contain half-sib crosses or those families with missing treatments (the "balanced model"). Further partitioning of variance components was possible with the balanced data set. Additional analyses were conducted for seed trees that were mated with half-sibs. Computations for estimating variance components followed Steel and Torrie (1960, Section 7.13).

The analysis of variance of the SMP study was in the form of a modified strip-plot design with nested effects (Petersen 1985, Section 10.4). Data were again unbalanced. Pollination methods (wind+blast vs. wind+blast+wand), floral phenology (early vs. mid vs. late season flowering), and crown levels, were considered treatment factors and fixed effects. Trees and cones were treated as nested and random effects (Table 7). Trees within families occasionally belonged to different phenological classes; therefore, analyses were not made for families or for trees within families.

Because SMP treatments were applied to entire trees (excluding

the isolated controls), treatment effects were evaluated by comparison of orchard relatives. Data collected from the SMP study trees were compared to responses of full-sib relatives in the inbreeding study. Open-pollinated collections from the inbreeding study trees had received natural wind pollination and the air blast treatment. These cones were contrasted with open-pollinated cones from the SMP study trees which had received wind pollination with both the air blast and the pollen wand treatments. SMP effects were also compared with the "wind only" cones (which had been isolated from all supplemental pollination treatments) and with the outcrossed cones of the inbreeding study.

RESULTS

PHENOLOGY PATTERNS AND CONE MORTALITY RATES

Production of male and female reproductive buds was moderate in 1987. Of the 31 families in the Vernonia-B block, 11 (31%) could be included in the study. Phenology records indicate that sampled trees were representative of the entire range of the block's flowering dates (personal communication, M. Bordelon, Oregon State Department of Forestry). Floral bud-burst was reached on April 2 for the earliest study tree, and on April 16 for the latest tree. The mean date that trees of the early-phenology class reached maximum receptivity was April 3, mean dates for mid- and late-phenology classes was April 9 and April 15, respectively.

Cone mortality was high and variable, ranging from 2% to 100% (mean = 46%, Table 5). Mortality was associated with individual trees (Table 5), but not with inbreeding treatments (self, full-sib, half-sib, and outcross pollinations) within trees.

Although numerous late-Spring frosts were noted, within-block temperature monitoring did not reveal any extremely low readings (Figure 4). A short duration moderately-low temperature of 29°F (-2°C) was recorded on April 19. Comparison between field notes

and developmental stages of aborted cones indicated that most damage occurred at approximately this time.

EFFECTS OF INBREEDING ON SEEDSET

Cone mortality resulted in many trees having fewer than five cones per treatment and, as noted earlier, half-sib pollen sources were available for only three study trees (Table 4). A completely balanced design would have included 400 cones (20 trees x 4 inbreeding levels x 5 cones); only 237 were available for analysis (Table 8). A total of 14,918 round seeds were produced (Table 9).

Per-cone seed potential (#flat+#empty+#filled+#insect damaged) was found to be highly variable (range = 48 to 116), and was significantly associated with families and trees within families (Table 10). Seed potential averaged 73.1 ± 1.58 across all cones and treatments.

Flat seeds can not be fertilized (Owens 1973), but previous observations have suggested a possible relationship between flat seed proportions and the degree of inbreeding (personal communication, F. Sorensen, USFS - PNW Experiment Station). Percent flat seed (PF) ranged from 4.9% to 50.6% per cone. The main effect of inbreeding

was not significant (mean values: $PF_{\text{self}} = 14.8$, $PF_{\text{full-sib}} = 13.9$, $PF_{\text{half-sib}} = 9.5$, $PF_{\text{outcross}} = 18.7$). Variation was primarily associated with trees within families, the interaction between inbreeding treatments and families, and experimental error (Table 10).

Variation in both filled and empty seed weights was primarily associated with families, trees within families, and experimental error; and was independent of inbreeding treatments (Table 10). Average filled seed weight was 14.24 milligrams (mg) and ranged from 10.04 to 16.77 mg between families, and from 14.50 to 18.85 mg between trees within families. Empty seed weights ranged from 5.33 to 8.17 mg between families, and from 5.67 to 8.33 mg between trees within families. Seed weights of bagged cones were not significantly different from those of open-pollinated collections (filled $wt_{\text{control-pol.}} = 14.21$ mg, filled $wt_{\text{open-pol.}} = 14.27$ mg, empty $wt_{\text{control-pol.}} = 6.49$ mg, empty $wt_{\text{open-pol.}} = 6.09$ mg).

Overall, inbreeding dramatically increased the proportion of empty seeds per cone, but families and trees within families, occasionally responded differently (e.g. trees 1-I#1 and 1-I#2, Table 11).

Outcrossing, the "optimal" type of pollination, produced an average

of 41.8 ± 2.7 percent empty seeds per cone ($n=74$ cones). Full-sib crosses resulted in 66.4 ± 2.5 percent empty seeds ($n=70$), while self pollinations led to a highly consistent 98.2 ± 0.2 percent empty seeds per cone ($n=81$) (Table 11). Relative yields of filled seeds with respect to that of outcrossed seeds, are as follows: self = 0.0314, full-sib = 0.5777, outcross = 1.0000.

On average, PE of half-sibs was less than that of full-sibs, although half-sib data represents only three crosses and 12 cones (Table 11). In two of the three cases where half-sib matings were possible, empty seed proportions for half-sibs were intermediate between those of outcrosses and full-sibs. These cases involved members of only one family (trees 25-I#1 and 25-I#2, Table 8). Average percent empty seeds for these two trees are as follows (numbers in parentheses are filled seed proportions relative to that of outcrossing): $PE_{\text{outcross}} = 31.7 \pm 6.2$ (1.000), $PE_{\text{half-sib}} = 38.6 \pm 3.5$ (0.899), $PE_{\text{full-sib}} = 65.1 \pm 2.9$ (0.511), $PE_{\text{self}} = 98.3 \pm 0.5$ (0.025). Because of cone mortality, there was no outcrossing data from the third case (tree 3-I#2, Table 8).

Analyses of variance of empty seed percents were performed on

transformed data sets of both the "full" and the "balanced" model. Because results were nearly identical, and because the full model contained such a large proportion of missing values, only results based on the balanced model are presented (Table 12). These data included only those individuals with two trees per family and no missing treatments. Values for the half-sib matings were also deleted.

As indicated by the raw data (Table 11), inbreeding treatments were the primary source of variation in seedset ($\theta^2_s = 69.3\%$ of total, $p < 0.0001$). Partitioning of this effect indicated a linear response, which was very highly significant (Table 12). The effect of increased empty seed proportions with increased inbreeding levels is depicted in Figure 5. Variation among trees within families also was highly significant ($\sigma^2_{d(f)} = 5.7\%$ of total, $p < 0.0001$), as was an interaction between inbreeding levels and trees within families ($\sigma^2_{d(f)s} = 5.5\%$ of total, $p = 0.0072$, Table 12). Variation attributable to other components was not significant.

Regression analyses were made with the previously described arcsine transformations of these seed data ($n=237$ cones). Regres-

sion of percent empty seeds per cone (PE), on inbreeding level (F), gave a very highly significant relationship ($p < 0.0001$, $R^2 = 0.66$, Figure 6). Back-transformed values indicate that between F (inbreeding coefficient) = 0.0 and F = 0.5, an increase in F of 0.1 is associated with an average increase of approximately 12% empty seeds per cone (arcsine squareroot $[PE/100\%] = 0.6518 + [1.5669 \times F]$). The percent empty seeds predicted by this model are as follows: outcross (F=0.0) = 36.60%, half-sib (F=0.125) = 56.21%, full-sib (F=0.25) = 74.68%, self (F=0.5) = 98.17%. The accompanying ratios of filled seed from inbreeding to filled seed from outcrossing are 1.000, 0.693, 0.401, and 0.029, respectively.

Regression analysis of PE data from the family with half-sib matings (family "25", Tables 8 and 11), produced results comparable to those of the entire data set (Figures 6 and 8). Predicted PE values were slightly lower than those of the larger model; but both regressions depict a positive, highly linear relationship with increased inbreeding. This highly significant model explained over 84% of the variation in PE ($R^2 = 0.8434$ $p < 0.0001$, Figure 8).

Level of inbreeding did not interact with families, but did reveal a significant non-linear interaction with trees within families

($p=0.0161$, Table 12), as well as indicating a nearly significant linear interaction between these same effects ($p=0.0551$, Table 12). Graphing of raw data indicated that the linear interaction was due to trees such as "1-I#2" and "4-I#2" (which formed near-linear, yet converging lines), while the non-linear interaction was due to trees such as "1-I#1" and "22-I#2" (which had lower PE values after full-sib mating than after outcrossing) (Table 11).

RESPONSE TO OPERATIONAL SMP TREATMENTS

The supplemental mass pollination study also had unbalanced data problems, primarily due to relatively few cones from the early and late phenology classes (Table 13).

Neither seed potential (average = 77.36 ± 0.82) nor flat seed proportions (average = 10.28 ± 0.25) were significantly affected by any of the tested effects. Insect damage was noted for $8.22 \pm 0.46\%$ of all seed, being highest in cones of the earliest flowering trees (early phenol. = $15.27 \pm 1.73\%$, mid = $6.97 \pm 0.48\%$, late = $7.20 \pm 0.91\%$).

Further inspection revealed several counter-intuitive results (Table 14). Averaged across all phenology classes and crown levels, "wind only" pollination produced an average of 44.1 percent empty

seed per cone. Wind pollination supplemented by the air blast treatment, produced an average of 51.5% empty seed per cone (a net increase of 7.4% empty seed); while the additional use of the pollen wand treatment, produced an average of 42.5% empty seed (1.6% less than that of wind pollination only). By comparison, regression analyses predicted an average of 36.6% empty seed per cone from controlled outcrossing, 56.2% from half-sib mating, 74.7% from full-sib mating, and 98.2% from self-pollination (Figure 6).

A rejection level of $\alpha = 0.10$ was used for the analysis, since there was considerable variation within 5-cone samples and frost damage had reduced many samples to less than the planned five. The intent of the larger alpha (0.10 instead of 0.05) was to avoid Type II errors.

Significant variation was associated with effect of phenology ($p = 0.0699$). The average percent empty seed was 33.8% across all early flowering trees, 47.5% for mid season, and 52.5% for those of late season flowering; thus, seedset was substantially higher in early flowering trees (Table 14). Early flowering wind pollinated cones also produced the highest seed yields (see "Wind Only", Table 14).

The "wind+blast" cones had a considerably higher average level of empty seed (51.5%) than did the "wind+blast+wand" cones (42.5%), although SMP treatment types were not statistically different ($p = 0.1085$). This lack of significance was primarily due to a large error term denominator for this test (trees within the phenology x SMP cells, Tables 7 and 15).

Non-significant variation was associated with the various Phenology x SMP Type combinations ($p = 0.9334$), while the effect of "trees within Phenology x SMP Type" was in turn very highly significant ($\sigma^2_{d(ps)} = 37.5\%$, $p < 0.0001$). Thus, effectiveness of SMP treatment was largely a function of individual trees, accounting for much more variation than the main effects of either phenology or SMP type.

PE was lowest in the upper crown regions (PE = 42.5%), while mid- and lower-crown empty seed proportions were higher and were not greatly different from each other (PE = 49.5% and 49.5%, respectively; Table 14). The main effect of crown levels (upper/middle/lower) was significant ($p = 0.0483$), while interactions of crown level with phenology, SMP treatment, or both phenology and SMP treatment were all non-significant. The interaction between crown

levels and trees within the phenology x SMP treatment cells was very highly significant ($p < 0.0001$), comprising 8.1% of the total variation in PE. This suggests that the quantity and mix (related/unrelated) of pollen that an individual orchard tree receives may be a function of specific crown characteristics.

DISCUSSION

EFFECTS OF INBREEDING

An extensive series of cone dissections revealed large variation in the number of total seed and flat seeds per cone. Analyzing treatment effects on the basis of the percent empty seeds per cone, rather than the more common use of the number of empty (or filled) seeds per cone, allowed for removing these confounding effects of variable numbers of total and flat seeds. Individual cone analyses are time consuming, although this approach also provides an indication of between-cone variability.

Seed potential ranged from 44 to 116 total seeds per cone, and was associated with both families ($p = 0.0884$) and trees within families ($p < 0.0001$) (Table 10). Considerable change in seed potential could apparently be accomplished through either purposeful or inadvertant selection for particular cone types.

Percent Flat Seeds

Percent flat seed per cone (PF) ranged from 1.1% to 74.2% among cones, and was primarily associated with cones within trees (error) and trees within families (Table 10). Although significant main

effects due to families or inbreeding treatments were not detected, an interaction between these two factors was significant. Graphing of raw data indicated that this interaction was primarily associated with cones of only one treatment (selfing) of one family. Flat seeds are formed prior to the time of ovule fertilization (Owens 1973), so shouldn't be affected by pollen treatments. Although my result could be due to sampling error, the observation that the PF interaction involved families, not trees within families, suggests that pollen may somehow influence ovule development prior to the time of fertilization.

Seed Weights

Seed weights were primarily associated with families and trees within families, and were independent of inbreeding treatments (Table 10). Significant inbreeding effects were not noted ($p < 0.10$); although when considering only the self, full-sib, and outcross pollinations (all but the 12 half-sib cones), filled seed weight is inversely proportional to the inbreeding coefficient (outcross = 14.54 mg, full-sib = 14.23 mg, self = 14.10 mg), and is therefore directly proportional to the number of filled seed per cone. This

result suggests that inbreeding may indeed have a slight depressing effect upon seed weights.

The embryo is the only inbred tissue within Douglas-fir seed (Owens 1973, Righter 1945). If all the difference between inbreeding treatments is due to differences in the embryo, inbreeding effects may be more important than is indicated by weighing entire seeds (Donaldson and Blackman 1974, Khalil 1981, Piesch and Stettler 1971). If embryos were to comprise 10% of filled seeds weights, the decrease of 0.44 mg would reflect 30% inbreeding depression in self-embryo weights ($14.54 \times 10\% = 1.454 \text{ mg} \approx$ out-cross embryo weight, $[1.454 - 0.44 \text{ mg}] / 1.454 = 0.70$).

Cone collections from natural timber stands average 11.55 mg per filled seed (Schopmeyer 1974); filled seed from this seed orchard weighed an average of 14.24 mg. Heavier seeds in the present study are assumed to be associated with the more optimal growing conditions of seed orchard trees. Studies in natural timber stands have also shown that warm temperatures inside isolation bags have a positive influence upon seed weights (Sorensen and Campbell 1985, Squillace 1957); the lack of such a relationship in this study may be associated with near-optimal growing conditions

of both bagged and un-bagged orchard cones.

Percent Empty Seeds

There was a strong positive relationship between inbreeding coefficients and percent empty seeds per cone. These results are consistent with those of previous Douglas-fir studies which have compared the effects of self-pollination to that of outcrossing (Orr-Ewing 1957, Piesch and Stettler 1971, Rehfeldt 1977, Sorensen 1971, Sorensen and Miles 1982). Similar effects of inbreeding are well documented for other conifers (Bramlet and Bridgwater 1986, Franklin 1970, Griffin and Lindgren 1985, Koski and Muona 1986, Plym Forshell 1953, Plym Forshell 1974, Sorensen 1970, Sorensen et al. 1976, Squillace and Kraus 1962), but the effects of low-level inbreeding are previously unpublished for Douglas-fir.

Self pollination of seed orchard trees gave a very high percentage of empty seeds per cone (PE = 98.2), higher than when similar techniques were applied in naturally occurring timber stands (PE = 89.2, Orr-Ewing 1957; PE = 91.5, Piesch and Stettler 1971; PE = 92.1, Sorensen 1971). This difference may be associated with factors such as the juvenility or the off-site planting of orchard

trees, and is not thought to be associated with differences in experimental technique. Seed orchards are customarily located on warmer, drier sites with different growing seasons than occur where the trees were customarily growing. Given the weakness of self embryos, these altered growing conditions may lead to increased environmental or maternal lethal effects (Bishir and Namkoong 1987).

Analyses of PE from the "balanced model" revealed an average relationship between $F = 0.0$ and $F = 0.5$ that is essentially linear (Table 12, Figure 6). Separate analyses for family "25" (the only family with data for all four levels of inbreeding treatments) also showed a highly linear relationship (Figure 8). Recent work in Canada (Woods and Heaman, unpublished. "Effect of different inbreeding levels in filled seed production in Douglas-fir". B.C. Ministry of Forests and Lands. Victoria, B.C.) also indicated a negative linear response between F and the number of filled seeds per cone.

Within family responses accounted for a highly significant proportion of the variation in seedset ($\sigma^2_{d(f)} = 5.71\%$ $p = 5.33 \times 10^{-5}$, Table 12). This variation is primarily due to non-linear responses of

certain trees ($D(F) \times s$, Table 12; e.g. trees 1-I#1 and 22-I#2, Table 11), although little of this variation is due to self-pollinations (Figures 5 and 7, Table 11). These results suggest the possibility of pollination technique problems, although this hypothesis can not be adequately tested until sampled trees include a wider range of self-fertilities.

PE of trees within families differed significantly in response to F, although the effect of families was itself non-significant ($p = 5.33 \times 10^{-5}$ vs. $p=0.4741$, Table 12). This may be a result of the small variation in PE after selfing, compared to the larger fluctuations in responses to the other inbreeding levels. Significant family effects may have been revealed had the sample included a larger variation in self-fertilities.

Natural Inbreeding

Levels of inbreeding under natural wind pollination may be estimated for the orchard block if it is assumed that all cones on a tree receive equal quantities of pollen (i.e. differences in empty seed proportions are due to inbreeding, not to lack of pollination). Seedling seed orchards do not have the potential for matings

between ramets of the same clone, but self, full-sib, and half-sib matings are possible. In this study, wind pollination produced an average of 44.07% empty seeds per cone (Table 14), while regression of controlled pollination data predicts a value of 36.60% from outcrossing (Figure 6). These differences suggest an average natural inbreeding level of $F \approx 0.047$ ($F = [\text{Arcsine}\sqrt{(44.07\%/100\%)} - 0.6518]/1.5669$, Figure 6). Results of an isozyme analysis in a clonal Douglas-fir seed orchard estimated an average inbreeding coefficient of this same value ($t=0.91$ $F=[1-t]/[1+t]=0.047$, Shaw and Allard 1982). The extensive family structure of the Vernonia-B block suggests that this study's value should be larger. That it is not, may be due to the young age of the trees, their lower pollen production, and the comparatively much lower self-fertility (which meant that most self-pollination resulted in empty seeds). A slightly higher estimate, $F=0.053$, has been reported for natural stands (El-Kassaby et al. 1981, Shaw and Allard 1982).

Effect on Genetic Gain

An important contrast between clonal and seedling orchards is in the types of inbreeding possible. Self-pollination is the only

inbreeding level in most clonal orchards, and results primarily in empty seeds. Seedling orchards, which can be composed of full-sib, half-sib, or open-pollinated families, have the potential for several levels of inbreeding.

Seedset from self pollination is extremely low, although previous Douglas-fir studies have indicated that percent germination of filled self pollinated seeds, is not greatly different from that of outcrossed seeds (Orr-Ewing 1957, Sorensen and Miles 1982). Subsequent extreme inbreeding depression in height, height growth, and survival of self pollinated seedlings has been reported (Orr-Ewing 1954, Orr-Ewing 1957, Sorensen and Miles 1982).

Compared with self-pollination, full- and half-sib pollinations produce high proportions of filled seeds (Table 11). The resulting seedlings are expected to show inbreeding depression in size, but it is not likely to be sufficient to permit removal of inbred seedlings through normal nursery culling procedures (Sorensen and Miles, 1982).

Using the linear relation between F and seedset, and by assuming linear relations between F and survival and between F and growth, it is possible to contrast the potential effects of natural inbreeding in

both clonal and seedling orchards. Self pollination ($F=0.5$) has been reported to cause 29% reduction in height growth and 17% reduction in survival at age 10 (Sorensen and Miles 1982). Interpolation for full-sib pollinations ($F=0.25$) predicts reductions of 14.5% for growth and 8.50% for survival. Similar predictions for half-sib matings ($F=0.125$) are 7.25% and 4.25%, respectively. Because no estimates of diameter reductions are available, it is assumed that inbreeding depression in diameter is proportional to that of height. It is also assumed that tree volume may be estimated by the volume of a cone ($\pi d^2 h / 12$), where d and h are the average diameter and height of trees resulting from outcross pollinations ($F=0.0$). The productivity of plantations resulting from non-inbred seedlings is set equal to 100%.

While polyembryony and pollen and embryo competition may occur when pollen is abundant (Fowler 1987, Sorensen 1982), one embryo per ovule and a lack of competition will be assumed for the purposes of these calculations. The relative productivity of 10 year old plantations may therefore be estimated from the equation:

$$\text{productivity} = (\text{volume of surviving outcrossed trees}) + (\text{volume of surviving inbred trees}) = ([100\% \text{ outcrossed plants}] - [\% F_x \text{ plants}]) + ([\% F_x \text{ plants}] \times [\text{Rel. Surv. } F_x]) \times$$

[Rel. Vol. F_x]

Where:

% F_x plants = percent of plantation established with inbred seedlings
of the given inbreeding coefficient

$$= (\%F_x \text{ Pol.})(\text{Rel. Seed. } F_x) / [(\%F_x \text{ Pol.})(\text{Rel. Seed. } F_x) + (1 - \%F_x \text{ Pol.})]$$

F_x = specified inbreeding level

% F_x Pol. = specified percentage of pollinations that are inbred

Rel. Seed. F_x = seedset of F_x relative to that of outcrossing

[Rel. Seedset $F_{\text{Self}} = 0.029$, $F_{\text{Full-sib}} = 0.401$, $F_{\text{Half-sib}} = 0.6929$, (see page 28)]

Rel. Surv. F_x = survival of F_x (at age 10) relative to that of outcrossing
[Rel. Survival $F_{\text{Self}} = 0.83$ $F_{\text{Full-sib}} = 0.915$ $F_{\text{Half-sib}} = 0.9575$]

Rel. Vol. F_x = tree volume of F_x (at age 10) relative to that of outcrossing
[Rel. volume $F_{\text{Self}} = (\pi(.71d)^2(.71h)/12)/((\pi d^2h)/12)^{-1} = 0.358$

$$F_{\text{Full-sib}} = (\pi(.855d)^2(.855h)/12)/((\pi d^2h)/12)^{-1} = 0.625$$

$$F_{\text{Half-sib}} = (\pi(.9275d)^2(.9275h)/12)/((\pi d^2h)/12)^{-1} = 0.798]$$

Algebraic manipulation generates the following single formula:

% productivity = $100\% \{ [100 - \%F_x \text{ Pol.}] / [(100 - \%F_x \text{ Pol.}) + (\%F_x \text{ Pol.})(\text{Rel. Seed. } F_x)] + [(\%F_x \text{ Pol.})(\text{Rel. Seed. } F_x)(\text{Rel. Surv. } F_x)(\text{Rel. Vol. } F_x)] / [(100 - \%F_x \text{ Pol.}) +$

$F_x)] + [(\%F_x \text{ Pol.})(\text{Rel. Seed. } F_x)(\text{Rel. Surv. } F_x)(\text{Rel. Vol. } F_x)] / [(100 - \%F_x \text{ Pol.}) +$

(%F_x Pol.)(Rel. Seed. F_x))

Therefore, assuming a hypothetical 20% self-pollination rate (i.e., the pollen cloud is composed of 20% self pollen/80% unrelated pollen), subsequent stand productivity (at age 10) would be estimated as follows:

$$\begin{aligned} \% \text{ productivity} &= 100\% \left(\frac{[100-20]}{[(100-20) + (20)(0.029)]} + \right. \\ &\left. \frac{[(20)(0.029)(0.83)(0.358)]}{[(100-20) + (20)(0.029)]} \right) \\ &= 99.49\% \\ &= 0.51\% \text{ productivity loss at age 10 due to 20\% natural self-pollination.} \end{aligned}$$

Similarly, a 20% full-sib pollination rate would produce plantations with 3.90% less volume than non-inbred stands, while 20% half-sib pollination would produce stands with 3.48% less volume than if all pollinations had been outcrosses.

Productivity estimates may be calculated for other combinations of inbreeding coefficients and inbred pollination rates. Whereas self-pollination primarily influences only seedset (Figure 9), full- and half-sib pollinations cause marked depressions in stand productivities (Figure 10). Higher seedset from half-sib pollina-

tions is compensated for by the lower survival and growth of full-sib pollinated seeds. The net result is only relatively minor differences in the effect of either type of sib-mating on stand productivity (Figure 10).

Self-pollination in natural stands has been previously reported to produce approximately 9% filled seeds per cone (Orr-Ewing 1957, Piesch and Stettler 1971, Sorensen 1971), and may be used as an estimate of the effects of inbreeding in a clonal orchard. This value is larger than that of the present study (1.8%), but if inserted into the above formula, still results in higher stand productivity than occurs with equivalent levels of half-and full-sib pollination (See " $F = 0.5$ ", Figure 10).

The average natural inbreeding level in the studied seed orchard has previously been shown to be $F \approx 0.047$; this being a result of some combination of selfing, full-sibbing, and to a lesser extent, half-sibbing. If, for the sake of example, this was due only to selfing ($F=0.5$) and outcrossing ($F = 0.0$), $F = 0.047$ would equate to a 77% self pollination rate. If all the natural inbreeding were due to only full-sib matings, the corresponding value would be 35% full-sib pollination.

Because of orchard age and male fecundity, it is unlikely that self-pollination would be nearly so common. It is probable that much of the inbreeding in this orchard was due to sib-mating (primarily full-sib mating), and therefore was of a type that would lead to some loss in stand productivity (Figure 10). A 10% to 20% rate of full-sib pollination may not be unlikely in this orchard block.

The above calculations involve use of an estimate of the average natural inbreeding level of this seed orchard. In addition to the potential imprecision of this estimate, it should be pointed-out that seeds are collected from individual orchard trees (not from "average" trees). For individual trees, values may widely differ from this mean inbreeding value.

Control of Inbreeding

Self pollination is most frequent in a tree's lower crown segments (El-Kassaby et al. 1986, Omi and Adams 1986). Omi (1985) proposed minimizing the collection of self-pollinated seeds by collecting cones from only upper crown regions of orchard trees. Avoidance of sib-mated seeds is not as simple.

Spacial proximity is one of many factors associated with

mating patterns; other components have been extensively discussed by other authors (El-Kassaby et al. 1984, El-Kassaby et al. 1988, El-Kassaby and Ritland 1986, Erickson 1987, Koski and Muona 1986, Moran and Griffin 1985, Sorensen 1972, Wheeler and O'Malley 1986). The likelihood of wind-pollinated mating is thought to be highest between trees that are phenologically synchronous, reproductively fecund, and spacially adjacent. Because related trees have a greater than random similarity in phenology, relatedness is a special problem in seedling orchards.

The probability of inbred matings may be minimized through the use of several managerial techniques. Bloom delay, via waterspray cooling treatments, may be effective in improving the diversity of the pollen cloud available to trees which otherwise would be phenologically early or late flowering (Silen and Keane 1969, El-Kassaby et al. 1988). Chemical fertilizers, plant hormones, and other physical treatments have been used to improve orchard mating patterns via floral stimulation of un-related trees (Philipson 1985, Ross et al. 1985, Silen 1973). Spacial orientation of orchard relatives may be accounted for at the time of orchard establishment, or may be altered through the use of specialized tree-moving

equipment (Marsh 1985, Silen and Wanek 1986). Under proper conditions, supplemental mass pollination (SMP) may also be effective in reducing inbreeding problems related to limited pollen availability (Daniels 1978).

EFFECTS OF SUPPLEMENTAL MASS POLLINATION

Neither operational SMP technique (blast nor blast+wand) produced seedset substantially greater than that of wind pollination alone (Table 14). Other seed orchards have indicated improved seedset via use of similar SMP methods (personal communication, D. Dotter, Weyerhaeuser Company), and successful results are reported in the literature (Brown 1987, Daniels 1978). Although the published techniques were quite similar to those used in this study, all other reports involved either different species, or clonal Douglas-fir orchards. Clonal orchards are generally established with scion material from older trees, and the upper crowns of these trees tend to be "open" down to the point of the graft union. Young trees from seed, however, usually have dense lower crowns which may intercept more self-pollen than the open crowns of clonal orchards.

Although not statistically significant, air blast SMP tended to decrease seedset from the level of wind-pollinated controls. This result is thought to be associated with an increased frequency of self-pollination and/or mating between commonly adjacent siblings (Figure 3). Although a beneficial response has been demonstrated in clonal orchards, it is not recommended that air blast SMP be used in seedling orchards without further testing.

Because this study was conducted within the operational practice of the Schroeder orchard, pollen wand SMP was used only in conjunction with the air blast treatment. Seedset may have benefited from the pollen wand treatment, although the confounding effects of air blast SMP make this difficult to assess. Pollen wand SMP may serve to alleviate pollen availability problems.

Although not statistically significant ($p = 0.2914$, Table 15), SMP treatment difference (blast minus blast+wand) decreased with increasing crown level ($\Delta_{(lower)} = 13.0\%$, $\Delta_{(mid)} = 8.0\%$, $\Delta_{(upper)} = 4.9\%$, Table 14). These results suggest that the air blast treatment may increase inbreeding (particularly selfing) in the lower crowns (where pollen catkins are concentrated). Additional use of the pollen wand may counteract this increase in PE.

Effect of Phenology

Seedset of both SMP regimes, as well as that of the wind pollinated controls, was highest in the earliest flowering trees (Table 14). Data from pollen monitoring devices suggested only minimal deposition of background pollen while early trees were flowering. Additionally, phenological records show that only a limited number of orchard trees were shedding pollen at this time. Filled seed proportions, however, suggest that pollen contamination was sufficient for pollination of early flowering trees. Proper interpretation of pollen monitoring data apparently requires better understanding of the relation between pollen deposition and ovule pollination rates. Seedset may also have been inconsistently depressed as a consequence of different potentials for sib-mating. Early flowering trees were related to 17% of the nearby pollen-donors (pollen-bearing trees within a 60 foot radius and within three days of synchronous reproductive maturity), mid- and late-phenology trees were related to 32% and 26% of their adjoining trees.

Effect of Crown Level

The highest average incidence of natural self pollination is reported to occur in the lower crown (El-Kassaby et al. 1986, Omi and Adams 1986). In the present study, the effect of crown level was evaluated only on supplementally pollinated cones. Under these conditions, PE was lowest in the upper crown and differed little between middle and lower crown ($PE_{(upper)} = 42.49$, $PE_{(middle)} = 49.46$, $PE_{(lower)} = 49.47$; Table 14). The effect of crown levels differed among trees ($p < 0.0001$), irrespective of both phenology ($p = 0.8636$) and SMP treatments ($p = 0.2914$). Trees such as 4-I#1 ($PE_{(upper)} = 37.12$, $PE_{(middle)} = 42.04$, $PE_{(lower)} = 40.50$) and 4-I#2 ($PE_{(upper)} = 31.82$, $PE_{(middle)} = 40.94$, $PE_{(lower)} = 49.04$) depict such a relationship. Field notes suggest that seedset may have been influenced by additional factors such as pollen production levels, the distribution of strobili throughout tree crowns, the proximity of related trees, and the synchrony of pollen dehiscence with the female receptivity of individual trees.

Significant variation was found between the seedset of specific trees, the phenology class to which they belong, and the SMP

treatments which they received (see "tree within (phenology x SMP)", Table 15). For example, trees 10-S#1 and 22-S#1 were both phenologically "late" and had each received the air blast and pollen wand SMP treatments, yet there was nearly 26% difference in PE values ($PE_{10-S\#1} = 57.89$, $PE_{22-S\#1} = 43.09$). The poorer response of tree 10-S#1 is thought to be associated with such factors as higher male fecundity, strobili distributed throughout the entire crown, near-synchronous phenology of its male and female flowers, and the the closer proximity of related trees.

CONCLUSIONS

In summation, control pollinations revealed a highly linear relationship between the inbreeding coefficient and the percent empty seeds per cone. Other traits, such as seed weights, percent flat seeds per cone, and seed potential, were not significantly influenced by level of inbreeding. A stand productivity model was developed which indicated that even though selfing was the worst form of inbreeding from a seed production standpoint, full-sibbing (and to a lesser extent, half-sibbing) caused the most pronounced inbreeding depression in stand productivity at ten years of age. The need for avoiding the deleterious effects of inbreeding, even in the form of sib-mating, is apparent.

The effectiveness of two operational SMP methods ("pollen wand" atomizer and tractor-drawn "air blaster") were also tested. Although previous studies have indicated beneficial responses from air blast SMP, results in this seedling seed orchard suggested that the air blast treatment was detrimental to seed yields. This was probably due to the air blaster increasing selfing rates in the dense crowns of seedling orchard trees.

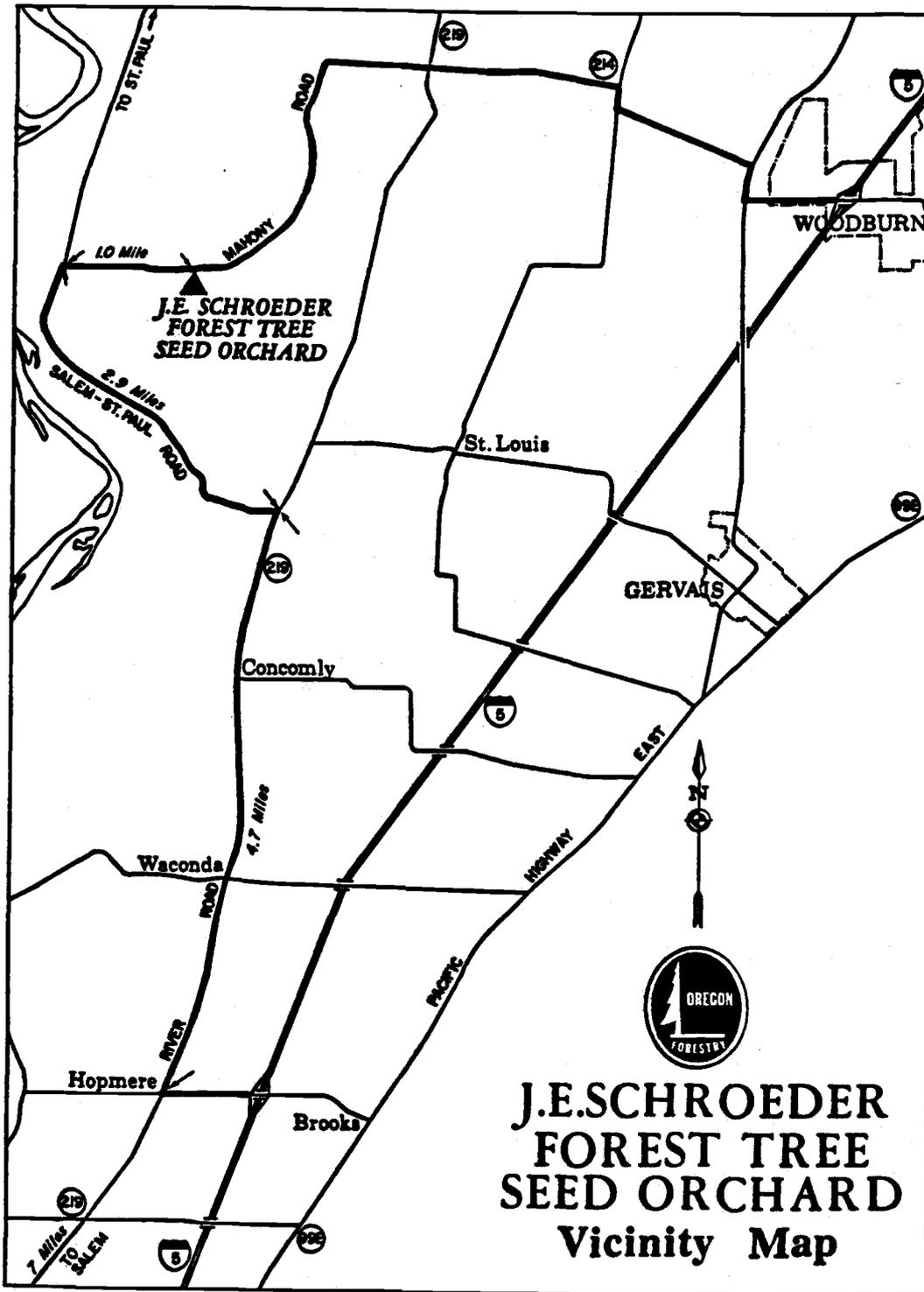


Figure 1. Location of the J.E. Schroeder Forest Tree Seed Orchard in the Willamette Valley north of Salem, Oregon.

J.E. SCHROEDER FOREST TREE SEED ORCHARD

PORTION OF SECS. 7 & 18 T.5S. R.2W. W.M. 8
PORTION OF SECS. 12 & 13 T.5S. R.3W. W.M.
MARION COUNTY, OREGON

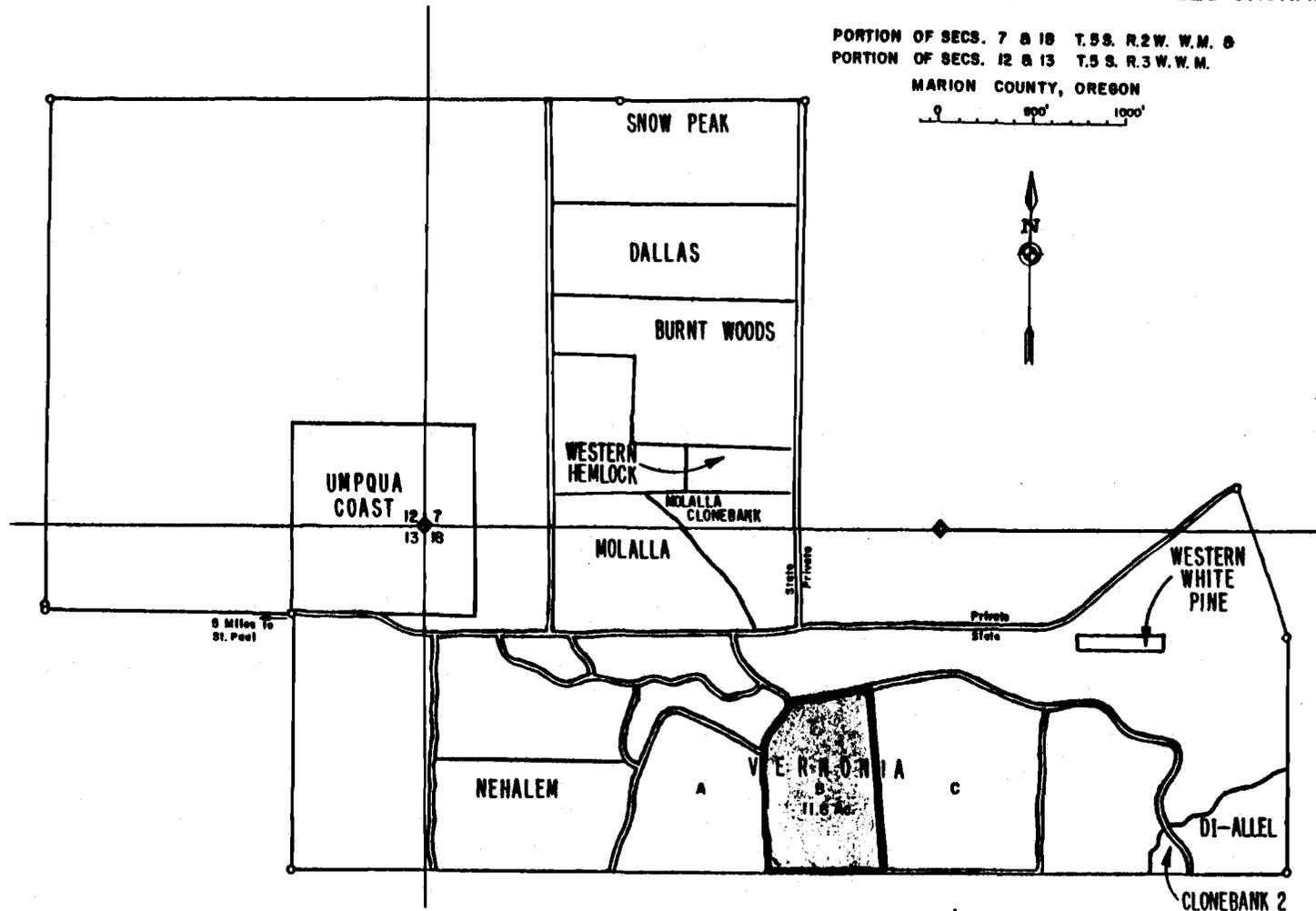


Figure 2. Map of the J.E. Schroeder Forest Tree Seed Orchard and location of the study block (shaded).

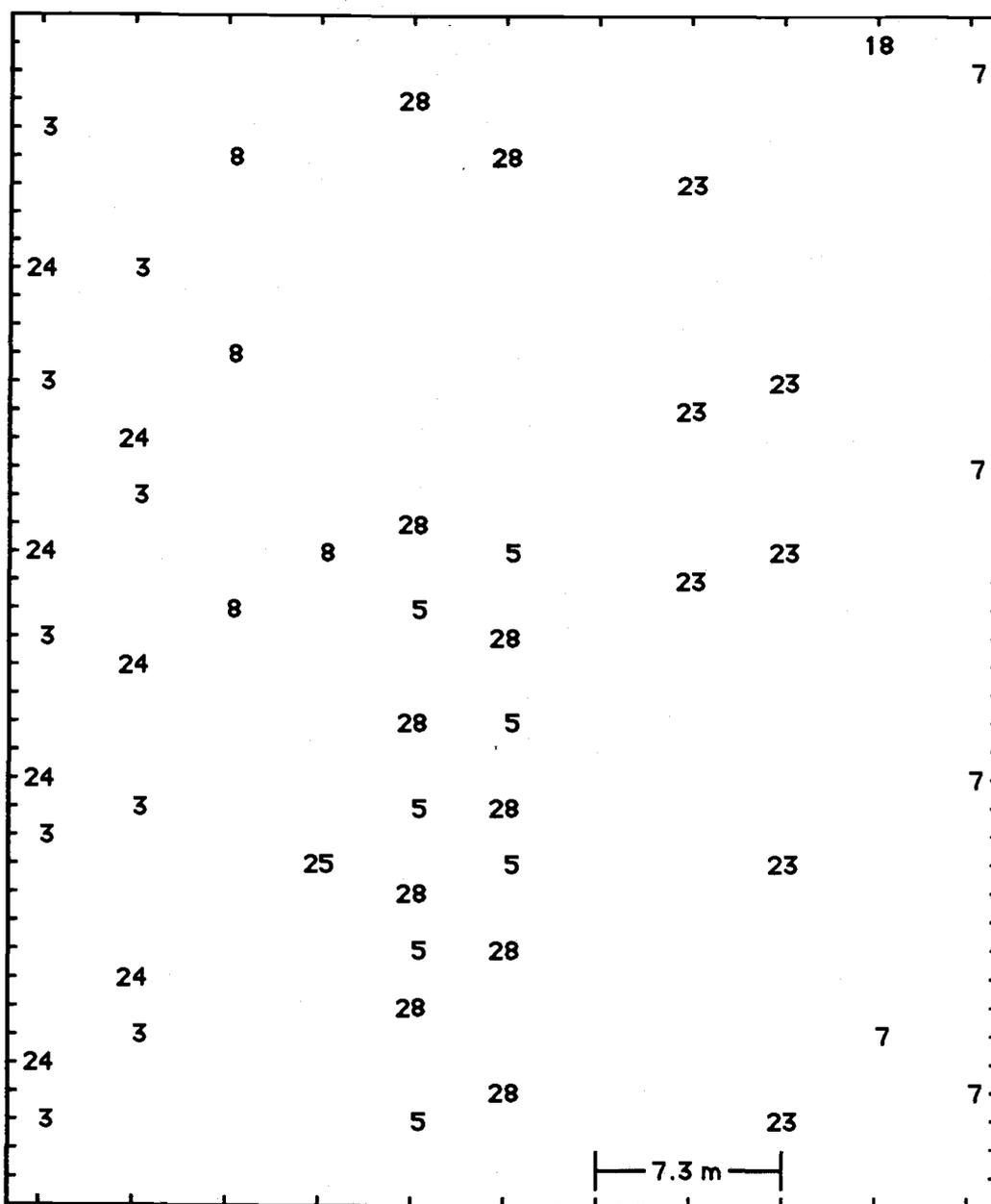


Figure 3. A 0.24 ha section of the Vernonia-B block illustrating uneven spacing and clumping of individuals from the same family (numbers refer to family designation of individuals at each locaiton).

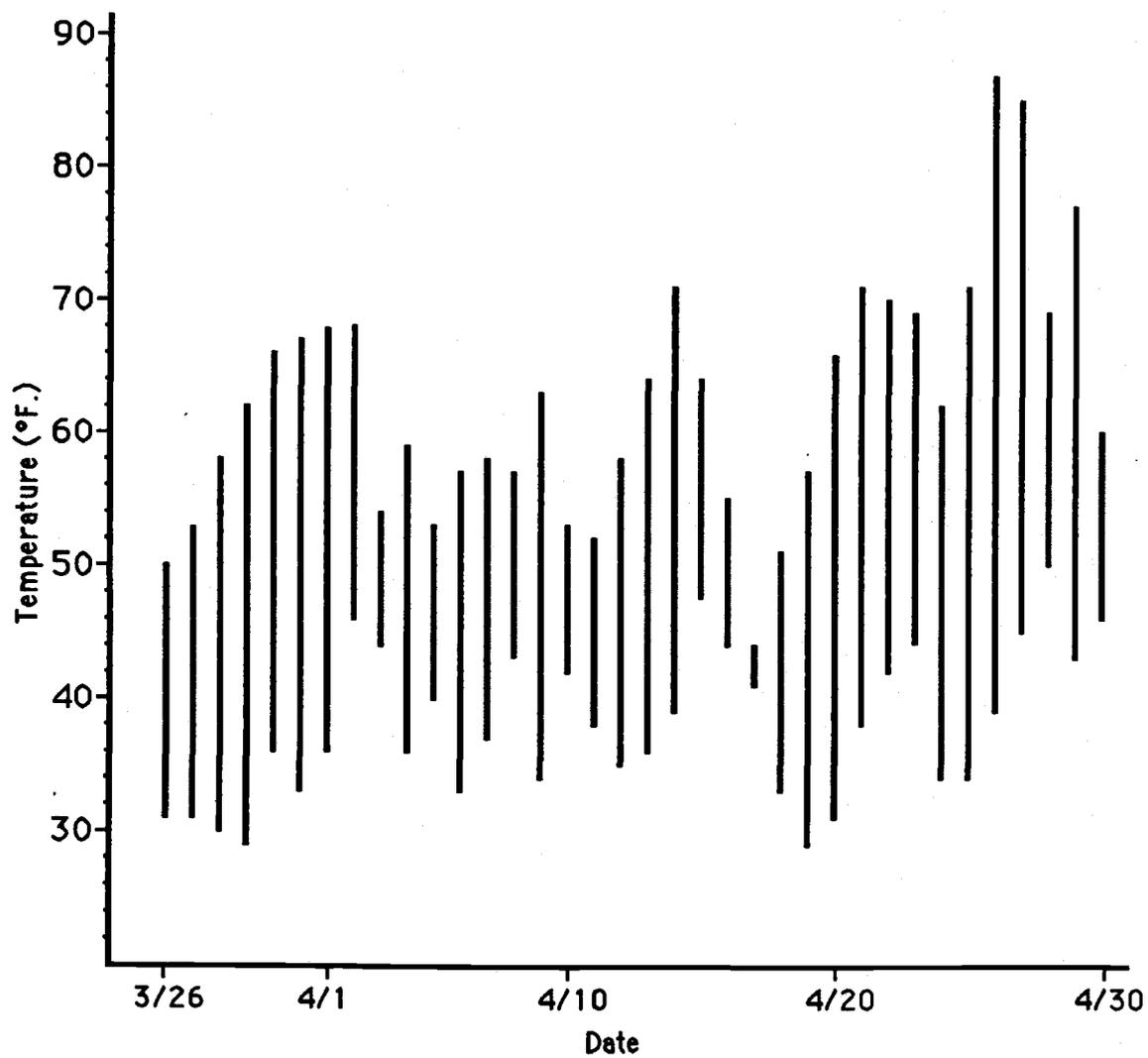


Figure 4. Daily temperature ranges in Vernonia-B block, in °F ($^{\circ}\text{C} = 5(^{\circ}\text{F} - 32)/9$).

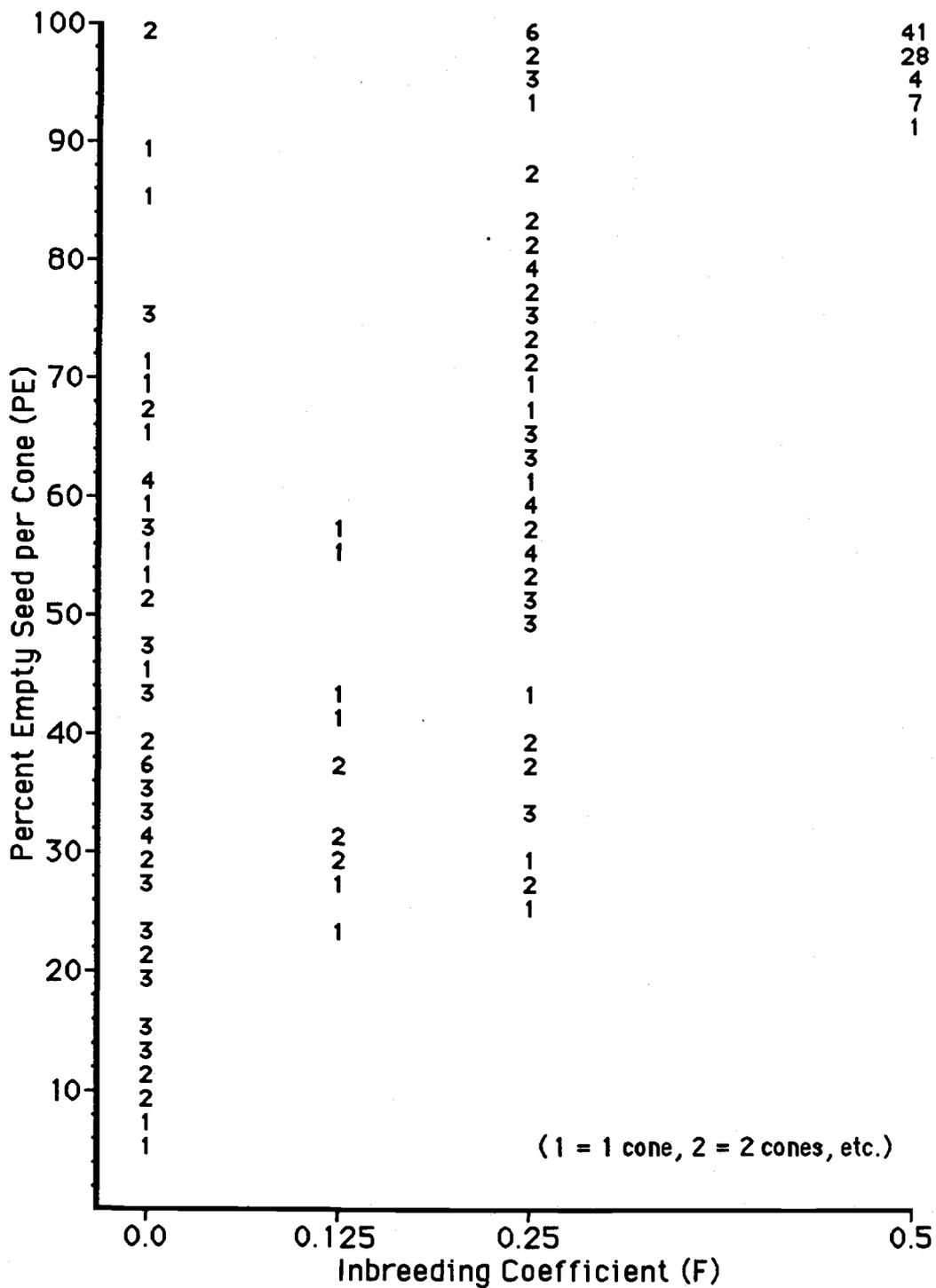


Figure 5. Percent empty seed per cone (PE) as inbreeding coefficient (F) increases from 0.0 to 0.5.

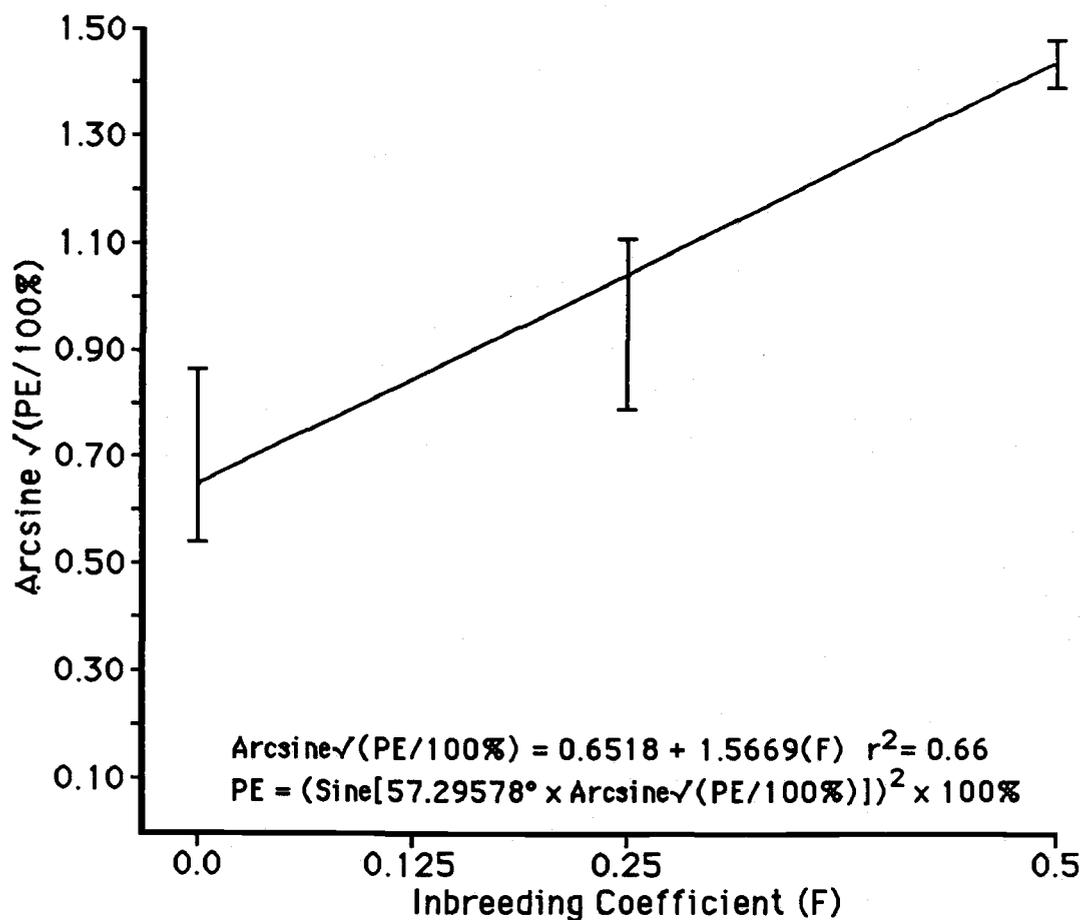


Figure 6. Response of transformed percent empty seed per cone (PE) as inbreeding coefficient (F) increases from 0.0 to 0.5, including both the regression model and observed standard errors of "Balanced Model" data.

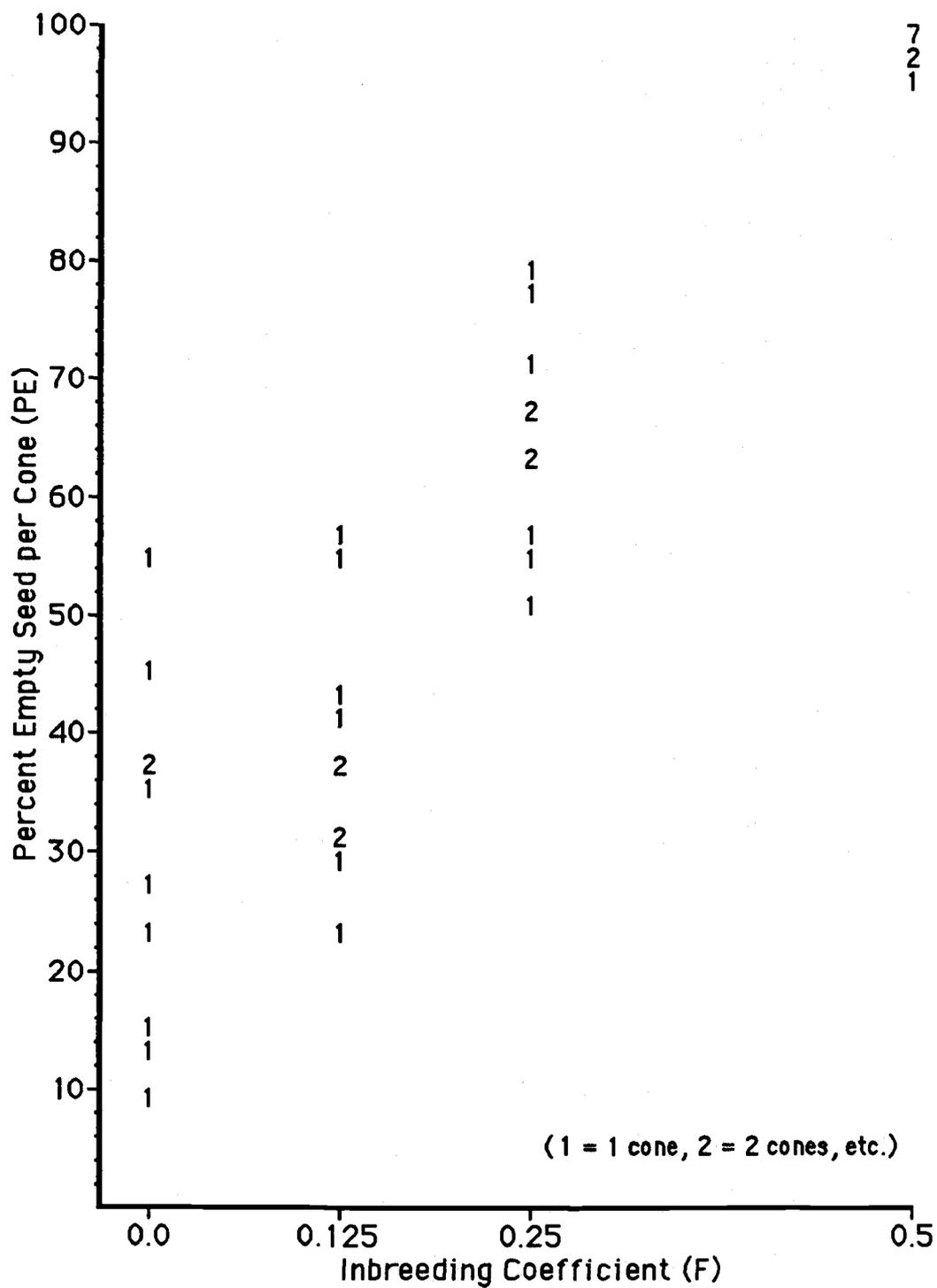


Figure 7. Percent empty seed per cone (PE) as inbreeding coefficient (F) increases from 0.0 to 0.5; values from family "25" only.

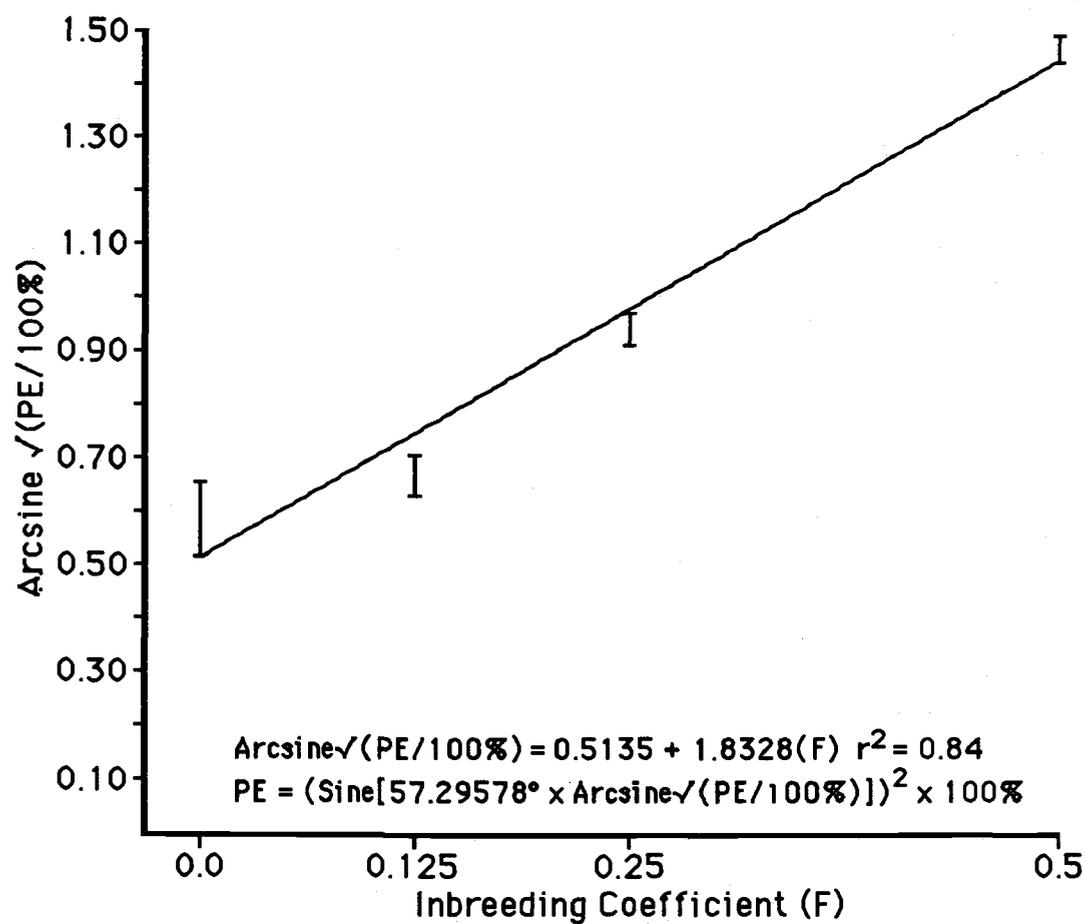


Figure 8. Response of transformed percent empty seed per cone (PE) as inbreeding coefficient (F) increases from 0.0 to 0.5, including both the regression model and standard errors of family "25".

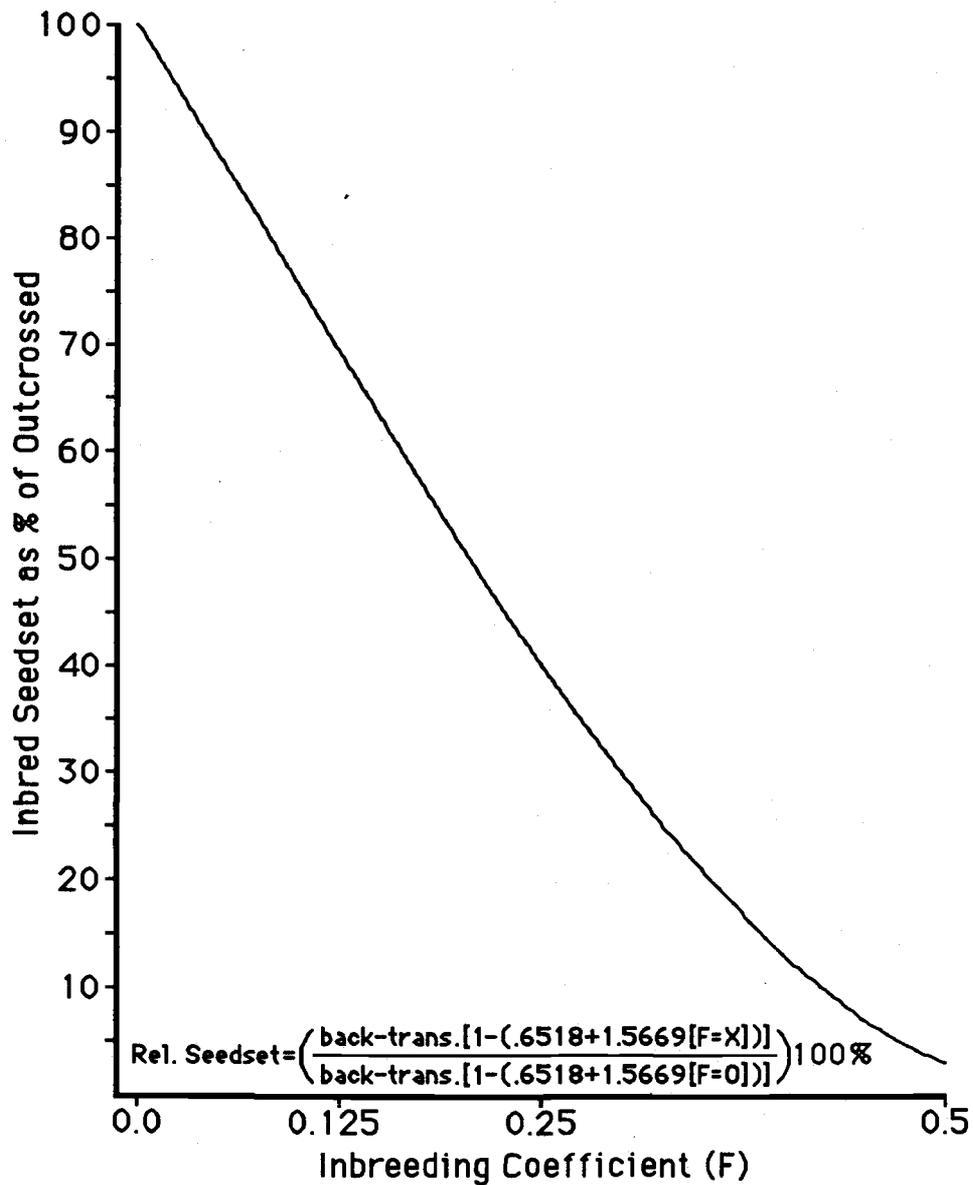


Figure 9. Relative seedset as inbreeding coefficients range from $F = 0.0$ to $F = 0.5$, values were back-transformed from the "Balanced Model" regression equation.

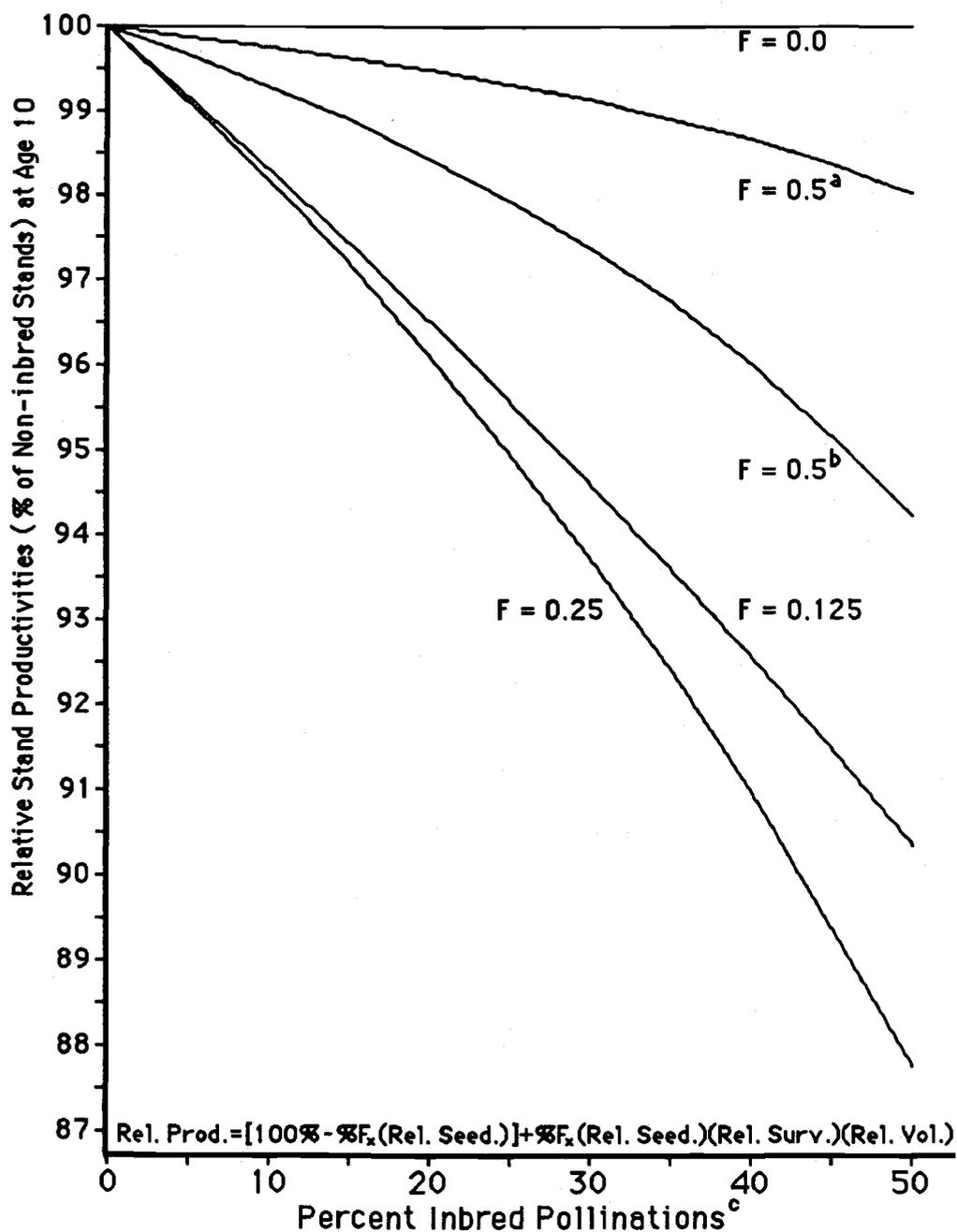


Figure 10. Estimated productivities of 10 year old stands resulting from between 0% and 50% inbred pollinations.

- ^a Stand productivities assuming 2.9% relative self-fertility (present study).
^b Stand productivities assuming 9.0% relative self-fertility (previous studies).
^c Twenty percent inbred pollination (abscissa) means that trees receive 20% self (or half-sib or full-sib) pollen and 80% genetically unrelated pollen.

Table 1. Mean times of maximum female receptivity for 31 full-sib families in Vernonia-B block, during 1983 and 1984.

| Family | Timing(83) ^a | Timing(84) | Z(83) ^b | Z(84) | Average Z | Trend ^c |
|--------|-------------------------|-------------------------------------|--------------------|-------|-----------|--------------------|
| 1 | 2.7 | 1.9 | -0.18 | -0.35 | -0.27 | MID |
| 2 | 3.3 | 2.4 | 0.60 | 0.37 | 0.49 | MID |
| 3 | 3.4 | 2.8 | 0.73 | 0.95 | 0.84 | LATE |
| 4 | 2.2 | 1.3 | -0.83 | -1.21 | -1.02 | EARLY |
| 5 | 2.8 | 1.6 | -0.05 | -0.78 | -0.42 | varied |
| 6 | 3.4 | 3.0 | 0.73 | 1.24 | 0.99 | LATE |
| 7 | 2.7 | 2.2 | -0.18 | 0.08 | -0.05 | MID |
| 8 | | (no yield '86 - exclude from study) | | | | |
| 9 | 2.6 | 1.3 | -0.31 | -1.21 | -0.76 | varied |
| 10 | 3.2 | 2.9 | 0.47 | 1.09 | 0.78 | varied |
| 11 | | (planted late - exclude from study) | | | | |
| 12 | 3.5 | 2.4 | 0.86 | 0.37 | 0.62 | varied |
| 13 | 3.4 | 3.1 | 0.73 | 1.38 | 1.06 | LATE |
| 14 | 2.3 | 1.5 | -0.70 | -0.92 | -0.81 | EARLY |
| 15 | 2.2 | 1.8 | -0.83 | -0.49 | -0.66 | varied |
| 16 | 1.6 | 1.2 | -1.61 | -1.36 | -1.49 | EARLY |
| 17 | 5.0 | 2.0 | 2.81 | -0.20 | 1.31 | varied |
| 18 | 3.1 | 2.6 | 0.34 | 0.66 | 0.50 | MID |
| 19 | 3.4 | 2.6 | 0.73 | 0.68 | 0.71 | LATE |
| 20 | 2.9 | 2.3 | 0.08 | 0.23 | 0.16 | MID |
| 21 | 1.9 | 1.3 | -1.22 | -1.21 | -1.22 | EARLY |
| 22 | 3.0 | 3.7 | 0.21 | 2.25 | 1.23 | varied |
| 23 | 2.2 | 1.5 | -0.83 | -0.92 | -0.88 | EARLY |
| 24 | 2.4 | 2.1 | -0.57 | -0.06 | -0.32 | MID |
| 25 | 3.1 | 2.4 | 0.34 | 0.37 | 0.36 | MID |
| 26 | 1.0 | 1.0 | -2.39 | -1.66 | -2.03 | EARLY |
| 27 | 3.8 | 3.2 | 1.25 | 1.53 | 1.39 | LATE |
| 28 | 2.8 | 1.7 | -0.05 | -0.64 | -0.35 | MID |
| 29 | 2.9 | 1.9 | 0.08 | -0.35 | -0.14 | MID |
| 30 | 3.5 | 2.8 | 0.86 | 0.95 | 0.91 | LATE |
| 31 | 2.0 | 1.6 | -1.09 | -0.78 | -0.94 | EARLY |

| Summary: | Year | Average Timing | Std. Dev. |
|----------|------|----------------|-----------|
| | 1983 | 2.8379 | 0.7688 |
| | 1984 | 2.1414 | 0.6936 |

a Timing = average time of maximum female receptivity, in four day periods from date of first observed flushing of female buds.

b Z = Z-Score = (family average - average across families)/(standard deviation across families)

c Trend = classification according to average Z-Score (Early≤-0.68, -0.68<Mid<0.68, Late≥0.68, varied= different "trends" between years)

Table 2. Mean seed yields for 31 full-sib families in Vernonia-B block, during 1985 and 1986.

| Family# | Yield ₍₈₅₎ ^a | Yield ₍₈₆₎ | Z ₍₈₅₎ ^b | Z ₍₈₆₎ | Average Z | Trend ^c |
|---------|------------------------------------|-------------------------------------|--------------------------------|-------------------|-----------|--------------------|
| 1 | 0.835 | 0.392 | 0.99 | 1.64 | 1.32 | HIGH |
| 2 | 0.877 | 0.279 | 1.26 | 0.30 | 0.78 | varied |
| 3 | 0.650 | 0.202 | -0.19 | -0.62 | -0.41 | MED |
| 4 | 0.976 | 0.249 | 1.90 | -0.06 | 0.92 | varied |
| 5 | 0.712 | 0.238 | 0.21 | -0.19 | 0.02 | MED |
| 6 | 0.787 | 0.304 | 0.67 | 0.59 | 0.64 | MED |
| 7 | 0.315 | 0.145 | -2.33 | -1.29 | -1.81 | LOW |
| 8 | 0.608 | 0.000 | (exclude from study) | | | -- |
| 9 | 0.813 | 0.123 | 0.85 | -1.55 | -0.35 | varied |
| 10 | 0.813 | 0.332 | 0.85 | 0.93 | 0.89 | HIGH |
| 11 | | (planted late - exclude from study) | | | | -- |
| 12 | 0.833 | 0.390 | 0.98 | 1.61 | 1.30 | HIGH |
| 13 | 0.621 | 0.277 | -0.37 | 0.27 | -0.05 | MED |
| 14 | 0.531 | 0.163 | -0.95 | -1.08 | -1.02 | LOW |
| 15 | 0.771 | 0.324 | 0.59 | 0.83 | 0.71 | varied |
| 16 | 0.723 | 0.255 | 0.28 | 0.01 | 0.15 | MED |
| 17 | 0.407 | 0.158 | -1.74 | -1.14 | -1.44 | LOW |
| 18 | 0.685 | 0.436 | 0.04 | 2.16 | 1.10 | varied |
| 19 | 0.742 | 0.209 | 0.40 | -0.53 | 0.07 | MED |
| 20 | 0.817 | 0.368 | 0.88 | 1.35 | 1.12 | HIGH |
| 21 | 0.399 | 0.136 | -1.79 | -1.40 | -1.60 | LOW |
| 22 | 0.511 | 0.178 | -1.08 | -0.90 | -0.99 | LOW |
| 23 | 0.628 | 0.213 | -0.33 | -0.48 | -0.41 | MED |
| 24 | 0.782 | 0.260 | 0.66 | 0.07 | 0.37 | MED |
| 25 | 0.557 | 0.187 | -0.78 | -0.79 | -0.79 | LOW |
| 26 | 0.601 | 0.249 | -0.50 | -0.06 | -0.28 | MED |
| 27 | 0.663 | 0.171 | -0.10 | -0.98 | -0.54 | varied |
| 28 | 0.782 | 0.282 | 0.66 | 0.33 | 0.50 | MED |
| 29 | 0.769 | 0.317 | 0.57 | 0.75 | 0.66 | varied |
| 30 | 0.584 | 0.339 | -0.61 | 1.01 | 0.20 | varied |
| 31 | 0.518 | 0.187 | -1.03 | -0.79 | -0.91 | LOW |

| Summary: | Year | Average Yield | Std. Dev. |
|----------|------|---------------|-----------|
| | 1985 | 0.6794 | 0.1564 |
| | 1986 | 0.2539 | 0.0843 |

^a Yield = pounds of seed per bushel of collected cones (1 lb./bushel= 12.87 Kg/m³).

^b Z = Z-Score = (family average - average across families)/(standard deviation across families).

^c Trend = classification according to average Z-Score (Early≤-0.68, -0.68<Mid<0.68, Late≥0.68, varied= different "trends" between years)

Table 3. Classification of test trees according to reproductive phenology and seed yields.

| Family# | Tree Code ^a | Phenology ^b | Yield ^c |
|---------|------------------------|-------------------------|-----------------------|
| 21 | I # 1 | EARLY | LOW |
| 9 | I # 1 | EARLY -MID ^d | MED-LOW ^e |
| 9 | I # 2 | EARLY-MID ^d | MED-LOW ^e |
| 9 | S#1 | EARLY-MID ^d | MED-LOW ^e |
| 9 | S#2 | EARLY-MID ^d | MED-LOW ^e |
| 23 | I # 1 | EARLY | MED |
| 23 | I # 2 | EARLY | MED |
| 23 | S#1 | EARLY | MED |
| 23 | S#2 | EARLY | MED |
| 4 | I # 1 | EARLY | MED-HIGH ^e |
| 4 | I # 2 | EARLY | MED-HIGH ^e |
| 4 | S#1 | EARLY | MED-HIGH ^e |
| 4 | S#2 | EARLY | MED-HIGH ^e |
| 25 | I # 1 | MID | LOW |
| 25 | I # 2 | MID | LOW |
| 25 | S#1 | MID | LOW |
| 25 | S#2 | MID | LOW |
| 5 | I # 1 | MID-LATE ^d | MED |
| 5 | I # 2 | MID-LATE ^d | MED |
| 5 | S#1 | MID-LATE ^d | MED |
| 5 | S#2 | MID-LATE ^d | MED |
| 1 | I # 1 | MID | HIGH |
| 1 | I # 2 | MID | HIGH |
| 1 | S#1 | MID | HIGH |
| 1 | S#2 | MID | HIGH |
| 22 | I # 1 | MID-LATE ^d | LOW |
| 22 | I # 2 | MID-LATE ^d | LOW |
| 22 | S#1 | MID-LATE ^d | LOW |
| 22 | S#2 | MID-LATE ^d | LOW |
| 13 | I # 1 | LATE | MED |
| 13 | I # 2 | LATE | MED |
| 13 | S#1 | LATE | MED |
| 13 | S#2 | LATE | MED |
| 3 | I # 1 | LATE | MED |
| 10 | I # 1 | MID-LATE ^d | HIGH |
| 10 | I # 2 | MID-LATE ^d | HIGH |
| 10 | S#1 | MID-LATE ^d | HIGH |
| 10 | S#2 | MID-LATE ^d | HIGH |

^a Inbreeding study trees indicated with "I", SMP study trees indicated with "S"

^b Family flowering pattern (see "Trend", Table 1)

^c Family seed yield patterns (see "Trend", Table 2)

^d Classified as "varied" in Table 1.

^e Classified as "varied" in Table 2.

Table 4. Controlled cross combinations made in the inbreeding study.

| Female ^a parent | Male parents for different inbreeding treatment levels | | | |
|-------------------------------|--|--------------------------------------|------------------------------------|--------------------------------|
| | Outcross ^b (F=0.000) | Half-sib ^{a c} (F=0.125) | Full-sib ^a (F=0.250) | Self ^a (F=0.500) |
| 1-I #1 | OC#2 | ----- | 1-I #3 | 1-I #1 |
| 1-I #2 | OC#3 | ----- | 1-I #4 | 1-I #2 |
| 3-I #1 | OC#2 | 12-I #1 | 3-I #3 | 3-I #1 |
| 4-I #1 | OC#1 | ----- | 4-I #3 | 4-I #1 |
| 4-I #2 | OC#1 | ----- | 4-I #4 | 4-I #2 |
| 5-I #1 | OC#3 | ----- | 5-I #3 | 5-I #1 |
| 5-I #2 | OC#3 | ----- | 5-I #4 | 5-I #2 |
| 9-I #1 | OC#1 | ----- | 9-I #3 | 9-I #1 |
| 9-I #2 | OC#2 | ----- | 9-I #4 | 9-I #2 |
| 10-I #1 | OC#3 | ----- | 10-I #3 | 10-I #1 |
| 10-I #2 | OC#3 | ----- | 10-I #4 | 10-I #2 |
| 13-I #1 | OC#1 | ----- | 13-I #3 | 13-I #1 |
| 13-I #2 | OC#3 | ----- | 13-I #4 | 13-I #2 |
| 21-I #1 | OC#1 | ----- | 21-I #3 | 21-I #1 |
| 22-I #1 | OC#3 | ----- | 22-I #3 | 22-I #1 |
| 22-I #2 | OC#3 | ----- | 22-I #4 | 22-I #2 |
| 23-I #1 | OC#2 | ----- | 23-I #3 | 23-I #1 |
| 23-I #2 | OC#3 | ----- | 23-I #4 | 23-I #2 |
| 25-I #1 | OC#2 | 31-I #1 | 25-I #3 | 25-I #1 |
| 25-I #2 | OC#3 | 31-I #2 | 25-I #4 | 25-I #2 |

^a Individual male or female parents are designated as "family # - individual tree #", such that female "1-I #1" x male "1-I #3" indicates a full-sib mating.

^b For outcrosses, 3 pollen mixes of unrelated males were utilized:

OC#1 = 0-I #1, 16-I #1, 28-I #1, 33-I #1, 65-I #1, 793-I #1

OC#2 = 0-I #1, 16-I #1, 16-I #2, 28-I #2, 33-I #1, 39-I #1, 49-I #1, 51-I #1, 65-I #1, 416-I #1

OC#3 = 0-I #1, 4-I #1, 7-I #1, 7-I #2, 20-I #1, 21-I #2, 28-I #2, 29-I #1, 33-I #1, 49-I #1, 51-I #1, 65-I #1, 416-I #1

^c Unavailable pollen sources indicated by "-----"

Table 5. Cone mortalities of inbreeding study trees.

| Tree Number | Number of Cones | | Mortality Rate |
|---------------|-----------------|------------|----------------|
| | Bagged | Collected | |
| 1-I# 1 | 58 | 30 | 48.3% |
| 1-I# 2 | 64 | 16 | 75.0% |
| 3-I# 1 | 82 | 6 | 92.7% |
| 4-I# 1 | 85 | 60 | 29.4% |
| 4-I# 2 | 65 | 57 | 12.3% |
| 5-I# 1 | 64 | 0 | 100.0% |
| 5-I# 2 | 72 | 35 | 51.4% |
| 9-I# 1 | 52 | 7 | 86.5% |
| 9-I# 2 | 24 | 3 | 87.5% |
| 10-I# 1 | 58 | 46 | 20.7% |
| 10-I# 2 | 63 | 52 | 17.5% |
| 13-I# 1 | 64 | 57 | 10.9% |
| 13-I# 2 | 38 | 7 | 81.6% |
| 21-I# 1 | 56 | 48 | 14.3% |
| 22-I# 1 | 57 | 52 | 8.8% |
| 22-I# 2 | 78 | 16 | 79.5% |
| 23-I# 1 | 88 | 79 | 10.2% |
| 23-I# 2 | 79 | 35 | 55.7% |
| 25-I# 1 | 92 | 69 | 25.0% |
| 25-I# 2 | 99 | 97 | 2.0% |
| Total: | 1338 | 772 | 57.7% |

Table 6. Generalized format of the analysis of variance in the inbreeding study.

| Source | df ^a | Expected Mean Squares ^b |
|---------------------|-----------------|--|
| Family [F] | f - 1 | $\sigma^2 + rs \sigma^2_{d(f)} + rsd \sigma^2_f$ |
| Tree(Family) [D(F)] | f [d(f)-1] | $\sigma^2 + rs \sigma^2_{d(f)}$ |
| Inbreeding Tmt. [S] | s - 1 | $\sigma^2 + r \sigma^2_{d(f)s} + rdf \theta^2_s$ |
| F x S | (f-1)(s-1) | $\sigma^2 + r \sigma^2_{d(f)s} + rd \sigma^2_{fs}$ |
| D(F) x S | f[d(f)-1](s-1) | $\sigma^2 + r \sigma^2_{d(f)s}$ |
| Pooled Error | ds(r-1) | σ^2 |
| Total | rds-1 | |

^a d(f) = number of trees per family
 f = number of families
 r = number of cones
 s = number of pollen types

^b σ^2_f = family variance
 $\sigma^2_{d(f)}$ = tree within family variance
 θ^2_s = inbreeding treatment effect, considered random for partitioning of components
 σ^2_{fs} = family x inbreeding treatment variance
 $\sigma^2_{d(f)s}$ = tree within family x inbreeding treatment variance
 σ^2_{ds} = tree x inbreeding treatment variance
 σ^2 = experimental error

Table 7. Generalized format of the analysis of variance in the supplemental mass pollination (SMP) study.

| Source | df ^a | Expected Mean Squares ^b |
|----------------------|-----------------|--|
| Phenology [P] | p-1 | $\sigma^2_{r(cxd)(pxs)} + cr \sigma^2_{d(ps)} + cdrs \theta^2_p$ |
| SMP Type [S] | s-1 | $\sigma^2_{r(cxd)(pxs)} + cr \sigma^2_{d(ps)} + cdpr \theta^2_s$ |
| P x S | (p-1)(s-1) | $\sigma^2_{r(cxd)(pxs)} + cr \sigma^2_{d(ps)} + cdr \theta^2_{ps}$ |
| Trees [D] in (P x S) | (d-1)(ps) | $\sigma^2_{r(cxd)(pxs)} + cr \sigma^2_{d(ps)}$ |
| Crown Level [C] | c-1 | $\sigma^2_{r(cxd)(pxs)} + r \sigma^2_{cd(ps)} + dprs \theta^2_c$ |
| C x P | (c-1)(p-1) | $\sigma^2_{r(cxd)(pxs)} + r \sigma^2_{cd(ps)} + drs \theta^2_{cp}$ |
| C x S | (c-1)(s-1) | $\sigma^2_{r(cxd)(pxs)} + r \sigma^2_{cd(ps)} + drs \theta^2_{cs}$ |
| C x P x S | (c-1)(p-1)(s-1) | $\sigma^2_{r(cxd)(pxs)} + r \sigma^2_{cd(ps)} + dr \theta^2_{cps}$ |
| C x D(P x S) | (c-1)(d-1)(ps) | $\sigma^2_{r(cxd)(pxs)} + r \sigma^2_{cd(ps)}$ |
| Pooled Error | (r-1)(cdps) | $\sigma^2_{r(cxd)(pxs)}$ |
| Total | cdprs-1 | |

- ^a c = crown levels
d = number of trees
p = number of phenological classes
r = number of cones
s = number of SMP types

- ^b θ^2_p = phenological variance
 θ^2_s = SMP type variance
 θ^2_{ps} = phenology x SMP type variance
 $\sigma^2_{d(ps)}$ = tree within (phenology x SMP type) variance
 θ^2_c = crown level variance
 θ^2_{cp} = crown level x phenology variance
 θ^2_{cs} = crown level x SMP type variance
 θ^2_{cps} = crown level x phenology x SMP type variance
 $\sigma^2_{cd(ps)}$ = crown level x tree within (phenology x SMP type) variance
 $\sigma^2_{r(cxd)(pxs)}$ = experimental error

Note: For partitioning of components, fixed effects were treated as random.

Table 8. Number of study cones analyzed per treatment for trees in the inbreeding study.

| Tree Number | Inbreeding Treatment levels | | | | Total Cones |
|-------------|-----------------------------|-----------------------|-----------------------|-------------------|-------------|
| | Outcross (F=0.000) | Half-sib (F=0.125) | Full-sib (F=0.250) | Self (F=0.500) | |
| 1-I #1 * | 4 | 0 | 5 | 5 | 14 |
| 1-I #2 * | 5 | 0 | 3 | 5 | 13 |
| 3-I #1 | 0 | 2 | 2 | 2 | 6 |
| 4-I #1 * | 5 | 0 | 5 | 5 | 15 |
| 4-I #2 * | 5 | 0 | 5 | 5 | 15 |
| 5-I #1 | 0 | 0 | 0 | 0 | 0 |
| 5-I #2 | 5 | 0 | 5 | 5 | 15 |
| 9-I #1 | 1 | 0 | 3 | 3 | 7 |
| 9-I #2 | 2 | 0 | 0 | 1 | 3 |
| 10-I #1 * | 5 | 0 | 5 | 5 | 15 |
| 10-I #2 * | 5 | 0 | 5 | 5 | 15 |
| 13-I #1 | 5 | 0 | 5 | 5 | 15 |
| 13-I #2 | 0 | 0 | 0 | 0 | 0 |
| 21-I #1 | 5 | 0 | 5 | 5 | 15 |
| 22-I #1 * | 5 | 0 | 1 | 5 | 11 |
| 22-I #2 * | 2 | 0 | 1 | 5 | 8 |
| 23-I #1 * | 5 | 0 | 5 | 5 | 15 |
| 23-I #2 * | 5 | 0 | 5 | 5 | 15 |
| 25-I #1 * | 5 | 5 | 5 | 5 | 20 |
| 25-I #2 * | 5 | 5 | 5 | 5 | 20 |
| Total: | 74 | 12 | 70 | 81 | 237 |
| Average: | 3.7 | 0.6 | 3.5 | 4.1 | 11.9 |

* Trees included in "Balanced Model"

Table 9. Total number of round seeds per treatment for trees in the inbreeding study.

| Tree Number | Inbreeding Treatment levels | | | | Tree Total |
|------------------|-----------------------------|-----------------------|-----------------------|-------------------|---------------|
| | Outcross (F=0.000) | Half-sib (F=0.125) | Full-sib (F=0.250) | Self (F=0.500) | |
| 1-1#1 | 133 | * | 334 | 341 | 808 |
| 1-1#2 | 331 | * | 250 | 423 | 1,004 |
| 3-1#1 | * | 125 | 112 | 142 | 379 |
| 4-1#1 | 338 | * | 384 | 363 | 1,085 |
| 4-1#2 | 336 | * | 352 | 329 | 1,017 |
| 5-1#1 | * | * | * | * | * |
| 5-1#2 | 192 | * | 337 | 319 | 848 |
| 9-1#1 | 29 | * | 113 | 84 | 226 |
| 9-1#2 | 149 | * | * | 54 | 203 |
| 10-1#1 | 343 | * | 310 | 345 | 998 |
| 10-1#2 | 289 | * | 285 | 286 | 860 |
| 13-1#1 | 275 | * | 354 | 334 | 963 |
| 13-1#2 | * | * | * | * | * |
| 21-1#1 | 315 | * | 299 | 262 | 876 |
| 22-1#1 | 337 | * | 268 | 303 | 908 |
| 22-1#2 | 190 | * | 78 | 394 | 662 |
| 23-1#1 | 268 | * | 250 | 292 | 810 |
| 23-1#2 | 288 | * | 269 | 290 | 847 |
| 25-1#1 | 281 | 338 | 325 | 291 | 1,235 |
| 25-1#2 | 282 | 314 | 290 | 303 | 1,189 |
| Total | 4,376 | 777 | 4,610 | 5,155 | 14,918 |
| Avg./Cone | 59.1 | 64.8 | 65.9 | 63.6 | 62.9 |

* Missing values

Table 10. Variance components (as percentages) from analyses of variance of seed numbers, proportions, and weights in the inbreeding study.

| Source | Seed Potential ^a | Flat Seed ^b | Filled Seed Weight ^c | Empty Seed Weight ^d |
|----------------------------------|-----------------------------|------------------------|---------------------------------|--------------------------------|
| Family [F] | 35.072* | 2.852 | 39.828* | 12.469 |
| Tree(Family) [D(F)] | 27.571*** | 23.265*** | 21.162*** | 27.941** |
| Inbreeding Tmt. [S] ^e | -0.520 | -2.715 | -0.526 | 0.117 |
| F x S | -0.957 | 17.732* | 1.231 | -0.962 |
| D(F) x S | -0.662 | 6.117 | -2.652 | not avail. ^f |
| Pooled Error | <u>37.582</u> | <u>52.749</u> | <u>40.957</u> | <u>60.435</u> |
| Total | 100.00% | 100.00% | 100.00% | 100.00% |

a Seed Potential = number flat seeds + number round seeds

b Flat Seed Proportion = (number flat seeds + seed potential)

c Filled Seed Weights = filled seeds per cone weighed to nearest milligram

d Empty Seed Weights = 10 empty seed samples (per treatment, per tree) weighed to nearest milligram

e For the purpose of these calculations, this effect was considered random.

f Due to sampling of empty seeds, this component is not testable.

* p < 0.1

** p < 0.01

*** p < 0.001

Table 11. Average percent empty seed per treatment for trees in the inbreeding study.

| Tree Number | Inbreeding Treatment levels | | | |
|-------------|-----------------------------|-----------------------|-----------------------|-------------------|
| | Outcross (F=0.000) | Half-sib (F=0.125) | Full-sib (F=0.250) | Self (F=0.500) |
| 1-1#1 | 40.30 | * | 32.46 | 97.64 |
| 1-1#2 | 55.96 | * | 84.27 | 97.60 |
| 3-1#1 | * | 28.00 | 25.95 | 98.55 |
| 4-1#1 | 32.90 | * | 77.74 | 98.94 |
| 4-1#2 | 35.90 | * | 68.20 | 99.40 |
| 5-1#1 | * | * | * | * |
| 5-1#2 | 43.20 | * | 59.84 | 97.70 |
| 9-1#1 | 31.00 | * | 43.03 | 96.00 |
| 9-1#2 | 57.05 | * | * | 98.10 |
| 10-1#1 | 36.70 | * | 96.74 | 100.00 |
| 10-1#2 | 57.54 | * | 85.26 | 97.38 |
| 13-1#1 | 34.96 | * | 58.06 | 97.82 |
| 13-1#2 | * | * | * | * |
| 21-1#1 | 22.12 | * | 47.42 | 99.22 |
| 22-1#1 | 43.22 | * | 53.70 | 96.42 |
| 22-1#2 | 71.60 | * | 65.40 | 99.00 |
| 23-1#1 | 63.22 | * | 95.74 | 97.74 |
| 23-1#2 | 39.46 | * | 66.98 | 98.28 |
| 25-1#1 | 26.90 | 30.42 | 71.98 | 99.22 |
| 25-1#2 | 36.52 | 46.84 | 58.22 | 97.36 |
| Overall: | | | | |
| Mean | 41.79 | 36.86 | 66.37 | 98.17 |
| Std. Error | 2.68 | 3.16 | 2.54 | 0.22 |
| Cones/tmt. | 74 | 12 | 70 | 81 |

* Missing values

Table 12. Balanced-model analysis of variance of percent empty seeds after inbreeding.^a

| Source | df | Mean Square | F-ratio | prob. > F |
|----------------------------------|------------|----------------------|---------|--------------------------|
| Family [F] | 5 | 0.20689 ^b | 1.035 | 0.4741 |
| Tree(Family) [D(F)] | 6 | 0.19993 ^c | 5.388 | 5.33 x 10 ⁻⁵ |
| Inbreeding Tmt. [S] | 2 | 8.00900 ^d | 78.585 | 9.57 x 10 ⁻¹¹ |
| S _(linear) | 1 | 15.99831 | 156.977 | 2.18 x 10 ⁻¹¹ |
| S _(non-linear) | 1 | 0.01969 | 0.193 | 0.6647 |
| F x S | 10 | 0.11645 ^e | 1.297 | 0.3302 |
| F x S _(linear) | 5 | 0.07023 | 0.782 | 0.5816 |
| F x S _(non-linear) | 5 | 0.16266 | 1.811 | 0.1851 |
| D(F) x S | 12 | 0.08981 ^f | 2.420 | 0.0072 |
| D(F) x S _(linear) | 6 | 0.07871 | 2.121 | 0.0551 |
| D(F) x S _(non-linear) | 6 | 0.10090 | 2.719 | 0.0161 |
| Experimental Error | <u>130</u> | 0.037119 | | |
| Total | 165 | | | |

^a This model included only families with full complements of crosses on both trees in the family. Angular transformations were performed prior to analysis.

^b $\sigma^2_f = 0.00025$ (0.121% of total)

^c $\sigma^2_{d(f)} = 0.01179$ (5.706% of total)

^d $\theta^2_s = 0.14312$ (69.271% of total) Note: Considered a random effect for these purposes.

^e $\sigma^2_{fs} = 0.00289$ (1.399% of total)

^f $\sigma^2_{d(f)s} = 0.01145$ (5.542% of total)

^g $\sigma^2 = 0.03711$ (17.961% of total)

Table 13. Number of supplemental mass pollination (SMP) study cones dissected by phenology class, crown level, and pollination method.

| SMP Method | Crown Level | Phenology | | | Total Cones |
|------------|-------------|-----------|-----|------|-------------|
| | | Early | Mid | Late | |
| Blast+Wand | Upper | 20 | 55 | 15 | 90 |
| | Middle | 14 | 55 | 15 | 84 |
| | Lower | 9 | 55 | 15 | 79 |
| | Subtotals: | 43 | 165 | 45 | 253 |
| Blast | Upper | 10 | 60 | 20 | 90 |
| | Middle | 9 | 61 | 20 | 90 |
| | Lower | 9 | 55 | 20 | 84 |
| | Subtotals: | 28 | 176 | 60 | 264 |
| Wind Only | Random * | 16 | 50 | 15 | 81 |
| Totals: | | 87 | 391 | 120 | 598 |

* Isolated branches were distributed throughout tree crowns, and were allocated in proportion to each tree's distribution of available cones.

Table 14. Empty seed percentages by phenology class, crown level, and pollination method in the supplemental mass pollination (SMP) study.

| SMP Method | Crown Level | Phenology | | | Average Values ^a |
|------------|---------------------|-----------|-------|-------|-----------------------------|
| | | Early | Mid | Late | |
| Blast+Wand | Upper | 30.03 | 41.50 | 44.47 | 39.54 |
| | Middle | 28.22 | 47.46 | 51.46 | 44.47 |
| | Lower | 28.22 | 43.48 | 49.46 | 42.49 |
| | Average: | 29.12 | 44.47 | 48.46 | 42.49 |
| Blast | Upper | 34.71 | 44.47 | 52.46 | 44.47 |
| | Middle | 38.56 | 53.46 | 58.42 | 52.46 |
| | Lower | 47.46 | 56.44 | 57.43 | 55.45 |
| | Average: | 40.52 | 51.46 | 56.44 | 51.46 |
| Wind Only | Random ^b | 26.43 | 49.46 | 48.46 | 44.07 |
| Overall: | Mean | 33.76 | 47.46 | 52.46 | |
| | Std. Error | 0.04 | 0.01 | 0.04 | |
| | # Cones | 87 | 391 | 120 | |

^a Averages are weighted by numbers of values per class, since calculated across entire classes.

^b Isolated branches were distributed throughout tree crowns, and were allocated in proportion to each tree's distribution of available cones.

Table 15. Analysis of variance of empty seed proportions in the supplemental mass pollination (SMP) study.^a

| Source | df | Mean Square | F-ratio | prob. > F | Variance Components ^b | |
|---------------------|-----|-------------|---------|------------------------|----------------------------------|---------------|
| | | | | | calculated | % of Σ |
| Phenology [P] | 2 | 0.68173 | 2.903 | 0.0699 | 0.00286 | 7.0 |
| SMP Type [S] | 1 | 0.64113 | 2.730 | 0.1085 | 0.00174 | 4.3 |
| P x S | 2 | 0.01622 | 0.691 | 0.9334 | -0.00280 | -6.9 |
| Tree [D] in (P x S) | 31 | 0.23481 | 11.119 | $<1.0 \times 10^{-30}$ | 0.01530 | 37.5 |
| Crown Level [C] | 2 | 0.11536 | 3.165 | 0.0483 | 0.00051 | 1.3 |
| C x P | 4 | 0.01167 | 0.320 | 0.8636 | -0.00048 | -1.2 |
| C x S | 2 | 0.04574 | 1.255 | 0.2914 | 0.00012 | 0.3 |
| C x P x S | 4 | 0.01283 | 0.352 | 0.8417 | -0.00091 | -2.2 |
| C x D(P x S) | 71 | 0.03645 | 1.901 | 6.58×10^{-5} | 0.00329 | 8.1 |
| Experimental Error | 397 | 0.02112 | | | 0.02112 | 51.8 |
| Total | 516 | | | | | |

^a Angular transformations were performed prior to analysis.

^b For the purpose of these calculations, fixed effects were considered random.

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