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The effects of clonal variability, crown position of cones, and top-pruning of ramets to reduce height growth, on 1) the proportion of viable self-fertilized progeny (s) and 2) the proportion of filled seeds (PF), were investigated in a 20 year-old Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) seed orchard. Cones were collected from the upper one-third and lower one-third crown positions of pruned and nonpruned ramets (three each) from six clones. Estimates of s were derived from a maximum likelihood procedure using data from 10 allozyme loci (PGMI, LAP1, LAP2, GOT2, GOT3, GLYDH, CAT, 6PGD, IDH, and DIA). The six clones sampled varied significantly ($P < 0.05$) both in $\hat{s}$ and $\widehat{PF}$, but estimates of PF and s were not significantly correlated among clones. On the average, seeds from the upper portion of the crown were lower in $\hat{s}$ (0.055) and higher in $\widehat{PF}$ (0.512) than seeds from the lower crown ($\hat{s}$ = 0.190, $\widehat{PF}$ = 0.381). Combined over crown positions, pruning appeared to have little effect on $\hat{s}$, while pruned ramets ($\widehat{PF}$ = 0.432) had only a slightly lower filled

seed proportion than nonpruned ramets ($\widehat{PF}$ = 0.461). A total crown estimate of the proportion of viable progeny due to self-fertilization from the six sampled clones was about 10 percent.

These results show that crown position of cones, top-pruning, and clonal variation are all factors which could influence orchard management practices and the utilization of orchard seeds. Although the average proportion of viable progeny resulting from self-fertilization in the seed orchard appeared to be no greater than that found in natural populations, information on clonal variation may be useful in roguing decisions. It may be desirable to remove clones which are consistently high selfers and low seed producers; or perhaps seed from these clones should be excluded from commercial collections. In addition, orchard managers may consider not collecting lower crown cones because of the lower seed yields and greater proportions of self seed found in the lower crown. In this study, the effects of top-pruning orchard ramets on seed set or selfing appeared to be minimal, but it remains to be seen whether there is a relationship between severity of top-pruning and seed quality or quantity.

SEED SET AND THE PROPORTION OF PROGENY DUE TO SELF-FERTILIZATION IN A DOUGLAS-FIR SEED ORCHARD

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SEED SET AND THE PROPORTION OF PROGENY DUE TO SELF-FERTILIZATION IN A DOUGLAS-FIR SEED ORCHARD

I. INTRODUCTION

Seed orchards are designed to produce large amounts of genetically superior seed for reforestation. However, if inbreeding is significant due to high rates of self-fertilization, both the seed quantity and the genetic quality of seed crops could be severely reduced (Woessner and Franklin 1973). It is important to measure selfing rates so that decisions regarding seed orchard designs and special management practices to reduce selfing (for example, supplemental mass pollination (Woessner and Franklin 1973, Squillace and Goddard 1982)) can be evaluated. However, only a few estimates of selfing rates in seed orchards have been reported (Squillace 1977, Adams and Joly 1980a, Squillace and Goddard 1982, Shaw and Allard 1982).

Estimating the proportion of viable progeny due to self-fertilization has been greatly facilitated by the use of electrophoretic techniques (Adams and Joly 1980a, Adams and Joly 1980b, Clegg 1980). In conifers, seed tissue contains both haploid (megagametophyte) and diploid (embryo) tissues. Thus, maternal genotypes can be revealed by scoring haploid tissue and the pollen contribution to each embryo (and information on the mating system) can be inferred by the analysis of embryonic tissue (Müller 1976). Furthermore, in many species, numerous codominant allozyme loci have

been resolved, making it possible to use multilocus techniques in the estimation of mating system parameters (Shaw and Allard 1981).

In this study, it was of interest to measure the proportion of viable progeny resulting from self-fertilization in a coastal Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) seed orchard, and in particular to examine the effects on this proportion of:

1) clonal variability,

2) crown position of cones, and

3) top-pruning to reduce height growth of seed orchard ramets.

In a recent paper, Shaw and Allard (1982) found that the proportion of viable selfed progeny varied considerably among clones in one Douglas-fir seed orchard, and there was only a small difference in selfing between seeds collected in the upper and lower crowns. We report on measures of these two factors from data collected in another Douglas-fir seed orchard. In addition, since leader pruning has been discussed as a means of reducing height growth to facilitate cone collection in Douglas-fir seed orchards (Copes 1973), we have estimated the effect of this practice on the proportion of viable selfs. Finally, since self-pollination in Douglas-fir generally leads to low self-embryo viability (Sorensen 1982), we have also estimated the degree to which each of the above three factors affects the proportion of filled seeds.

In his literature survey of inbreeding depression, Franklin (1970) reported that for four genera of Pinaceae (Pinus, Picea, Larix, and Pseudotsuga) the average ratio of empty seeds after

self-pollination relative to cross-pollination was about 2:1. Among 30 clones in a Pinus sylvestris seed orchard, the average percentages of empty seeds were four to five times greater after self-pollination compared to open- and cross-pollination (H. Johnson, in Hadders and Koski 1975). Sorensen (1970, 1971) defined self-fertility in relative terms as the ratio of filled seeds after selfing to filled seeds after controlled cross-pollination. He found the average relative self-fertility to be 37 percent in Pinus ponderosa and 11 percent in Douglas-fir. Decreases in seed yield after selfing have also been reported in many other Douglas-fir studies (Duffield 1950, Orr-Ewing 1954, Orr-Ewing 1957, Sziklai 1966, Piesch and Stettler 1971, Rehfeldt 1978), with estimates of self-fertilities of individual trees ranging from nearly 0 percent to over 60 percent.

Reduced growth or vigor of selfed individuals in comparison to outcrossed progeny has been observed in most coniferous species (e.g., Franklin 1970, Hadders and Koski 1975, Squillace and Goddard 1982), although the effects can vary with age (Sorensen and Miles 1974, Sorensen and Miles 1982). Seed germinability of selfed seeds has been about 7 to 13 percent below germinability of cross-pollinated seeds for several genera in Pinus (Bingham and Squillace 1955, Franklin 1970). Inbreeding depression (the reduction of a metric character in self compared to cross-pollinated progenies) in survival after several years in field tests averages about 19 percent for several coniferous species (see Table 4 in Sorensen and Miles 1982). In addition, height depressions have been observed quite frequently. For example, in Pinus monticola there was an inbreeding

depression of 11 percent in first year nursery heights, which increased to 21 percent in both the second and third years (Bingham and Squillace 1955). Likewise, Sorensen and Miles (1974) recorded inbreeding depressions in height of 21, 26, and 32 percent for the first three years of growth in Pinus ponderosa. In a separate study (Sorensen and Miles 1982), the height depression was 36 percent after 10 years. Average inbreeding depressions (relative to open-pollinated progenies) of 28, 22, and 59 percent were found, respectively, for height, diameter, and average trunk volume in 61 year-old selfed Picea abies (Table 1 and Figure 6, Eriksson et al. 1973).

Similar reductions in seed germination, survival, and growth rate of selfed individuals have been observed in Douglas-fir (Allen 1942, Orr-Ewing 1954, Orr-Ewing 1957, Rehfeldt 1978, Sorensen and Miles 1974, Sorensen and Miles 1982). Sorensen and Miles (1974, 1982) found a 4 percent reduction in percent germination in var. menziesii. Inbreeding depression in seedling survival on the other hand, was 15.6 after the first two years and increased an additional 1.1 percent by age 10. Furthermore, they estimated inbreeding depressions in first, second, and ten year heights, of 18, 28, and 29 percent, respectively. Height depression of selfed seedlings was 32 percent after four years in var. glauca (Table 2, Rehfeldt 1978).

Because selfing can lead to severe depression in the fertility of parent trees and in growth of their progeny, it is important to determine the degree to which selfing occurs in natural populations and seed orchards. Sarvas (1962), using a method involving effective pollen catch, estimated that 26 percent of all pollinations in stands

of Pinus sylvestris were due to selfing. However, quantifying the actual proportion of ovules fertilized by self pollen is difficult due to the presence of polyembryony in many tree species, especially conifers (Sorensen 1982), and because self-pollination frequently results in nonviable (i.e., empty) seeds. Proportions of wind-pollinated seeds (both viable and nonviable) resulting from self-fertilization, though, have been estimated by comparing filled seed percentages, or proportions of viable progeny with gene markers after self-, wind-, and cross-pollinations. These estimates have ranged from 7 percent in the upper crowns (34 percent in the lower) of Pinus taeda (Franklin 1971a, Franklin 1971b) to 9.5 percent for 12 Pinus elliottii seed orchard clones (Squillace and Goddard 1982).

A more commonly measured parameter of selfing in wind-pollinated progenies of forest trees has been the proportion of viable progeny resulting from self-fertilization. This proportion has been determined through the detection of simply inherited marker alleles such as those coding chlorophyll deficiencies (Squillace and Kraus 1963, Sorensen 1973, Squillace and Goddard 1982), monoterpenes (Squillace 1977, Squillace and Goddard 1982), or allozymes unique to individual parent trees (Rudin and Lindgren 1977, Adams and Joly 1980a, Shen et al. 1981). Another technique is the analysis of allozyme frequencies in wind-pollinated progenies using single- and multiple-locus models (Mitton et al. 1977, Moran et al. 1980, Shaw and Allard 1981, Shaw and Allard 1982). Regardless of method used, however, estimates of the average frequency of viable selfs in seed crops of most commercially important tree species have generally been less than 10 per

cent. Exceptions are estimates in Eucalyptus species (23 and 37 percent selfs; Phillips and Brown 1977, Moran and Brown 1980), or in studies in conifers, where estimates are based on only a single or few trees (range 15 to 26 percent selfs; Cram 1960, Fowler 1965, Müller 1976).

The proportion of progeny due to selfing is of special interest in seed orchards because self-pollination can arise not only from pollination within a single ramet, but also from pollination which occurs between ramets of the same clone. However, there is no indication thus far that the potential for increased self-pollination is actually met in seed orchard crops. In three cases, estimates of viable selfs in seed orchards approximated those found for wild stands. Squillace (1977) estimated the proportion of selfs in a Pinus elliottii seed orchard to be 8.4 percent (an estimate of 6 percent was found in a natural stand (Squillace and Kraus 1963)). Adams and Joly (1980a) estimated the average frequency of viable selfs from five Pinus taeda seed orchard clones to be 1.2 percent (1.75 percent was found in a natural stand (Franklin 1968)). Shaw and Allard (1982) reported an average of 9 percent viable selfed progeny in a Douglas-fir seed orchard (estimates in natural stands ranged from 7 percent (Sorensen 1973) to 10 percent (Shaw and Allard 1982)). The average frequency of viable self-fertilized seed from 12 clones in eight different Pinus elliottii seed orchards (2.5 percent over a five year period) (Squillace and Goddard 1982) was quite a bit lower than that found by Squillace and Kraus (1963) for a natural population of this species (6 percent).

While average estimates of the proportion of self seedlings in seed orchards or natural stands are often less than 10 percent, considerable variation can occur among the progenies of individual clones or wild trees. The proportion of selfed progeny among individual trees in natural populations varied between 0 and 27 percent for both Pinus elliottii (Squillace and Kraus 1963) and Douglas-fir (Sorensen 1973). Squillace and Goddard (1982) found the range in frequency of viable selfed progeny to be 0.1 to 6.5 percent among 12 Pinus elliottii seed orchard clones (averaged over five years). In a Douglas-fir seed orchard, frequencies of selfed offspring among the progenies of five clones suspected of being high selfers (based on an earlier limited sample), were never less than 21 percent, and were never greater than 5 percent among the progenies of four clones suspected of being high outcrossers (Shaw and Allard 1982).

Individual wind-pollinated trees or seed orchard clones appear to vary in seed set as well. Filled seed percentages among six Douglas-fir trees in natural stands varied from 24.5 to 68.2 percent (Orr-Ewing 1957). In natural stands of Pinus ponderosa, Sorensen (1970) found filled seed percentage to range from 41.3 to 87.1 percent among 19 trees. In two years of sampling in a Pinus sylvestris seed orchard (Hadders 1971), filled seed percentages in the upper crown levels of ramets from 15 clones varied between 75 to 97 percent, while the corresponding range in the lower crown was approximately 45 to 95 percent (Tables 1 and 2, Hadders 1971).

Due to the distribution of male and female flowers in the crowns of monoecious tree species (Denison and Franklin 1975), seed set and

proportions of progeny due to selfing may also be influenced by crown position. In a young tree, the female flowers tend to occur primarily in the upper crown and male flowers in the mid and lower crown; however, with increasing age, male and female flowers become more closely associated in the lower and mid-crown regions, and self-pollination could subsequently be increased in these parts of the crown (Fowler 1965, Shen et al. 1981, Shaw and Allard 1982, Squillace and Goddard 1982). This may lead to seed set being less in cones from the lower crown than from the upper, although self-compatibility factors and pollen availability also influence filled seed percentages (Hadders 1971, Shaw 1980).

Actual results seem to bear out the expected relationship between seed set and crown position of cones. In two consecutive years of sampling in a 12 year-old Pinus sylvestris seed orchard, Hadders (1971) found the average percentage of empty seeds in upper crowns of ramets from 15 clones (12.7 percent) was less than the lower crown (27.2 percent). Likewise, from a study based on 12 Pinus elliottii seed orchard clones, Squillace and Goddard (1982) showed that empty seed percentages consistently increased in cones sampled from the upper to the lower parts of the crown (upper mean = 18.8, mid-upper mean = 23.2, mid-lower mean = 23.4, lower mean = 25.9).

Substantially higher frequencies of viable self seeds in cones collected from lower crowns than in upper crowns support the contention that higher rates of self-fertilization in lower crowns is a primary factor in reducing seed set. For example, among three Pinus banksiana trees, the average proportion of viable selfs was 13

percent in seeds from the upper crown and 26 percent in lower crown seeds (Fowler 1965). For six natural Douglas-fir populations, Shaw and Allard (1982) found that viable self frequencies averaged 7 and 14 percent in seeds from the upper and lower crowns respectively.

As might be expected, similar results have been reported for seed orchard clones. Shen et al. (1981) showed that the average proportion of viable self seeds from the upper crown (2.9 percent) was less than the lower (8.5 percent) for one Pinus sylvestris seed orchard clone. Although Squillace and Goddard (1982) did not convert the frequency of mutant seedlings to estimates of the frequency of viable selfed progeny, the average percentage of mutant seedlings from the lower (1.09) to the upper (0.45) crowns of seven Pinus elliottii seed orchard clones decreased significantly, and suggested that self-pollination was greater in lower crowns than upper crowns. However, only a small difference in the proportion of viable selfed progeny was observed between the upper (8 percent) and lower (11 percent) crowns in a Douglas-fir seed orchard (Shaw and Allard 1982).

Limiting crown height for ease of cone collection, as well as for convenience in flower induction treatments, insect and disease control, and controlled pollination are all factors which have led to the suggestion that top-pruning of orchard ramets could be a useful management tool in seed orchards (van Buijtenen and Brown 1963, Copes 1973, Gansel 1977). Previous studies have concentrated almost exclusively on the effect of top-pruning on strobili or cone production. Removal of the upper one-half of the crown of trees in a mature Pinus taeda plantation resulted in a 52 percent reduction in female strobili

relative to controls, while pruning to a height of five feet in a younger (five-year-old) plantation resulted in nearly a 100 percent reduction in female strobili (van Buijtenen and Brown 1963). McLemore (1979) found that there was no average difference in cone production between pruned and control Pinus taeda seed orchard clones (10- to 12-year-old grafts) when the data were combined over five years, but in one particular year, there was a 38 percent decrease in cone yield for the pruned trees. Likewise, for the younger clones (five- to six-year-old grafts), control trees were two to four times more productive than pruned trees in three of six years, but when the data were combined over six years, there was no difference. Gansel (1977) concluded that pruning Pinus elliottii seed orchard clones decreased cone production slightly, and Copes (1973) showed that cone yield decreased (34 to 39 percent) after leader pruning of six- to eight-year-old Douglas-fir seed orchard grafts. However, estimated total cone production on pruned Picea glauca clones (18 year-old field grafts) was 24 percent more than on untreated clones (Nienstaedt 1981). It is difficult to compare the results of these studies not only because different species were involved, but also because experiments differed in tree age, time of year of pruning, and the degree of top-pruning. Although Fowler (1965) suggested that manipulation of tree crowns in seed orchards could bring the male and female flowers closer together and therefore increase the potential for self-pollination, there have apparently been no studies investigating the effect of top-pruning on viable selfed progeny production or seed set.

II. MATERIALS AND METHODS

II.1. Description of orchard and sampling

The Douglas-fir clones utilized in this investigation are located in the 21 hectare David T. Mason Seed Orchard, managed by Barringer and Associates (Sweet Home, Oregon). Initiated in 1960, this is one of the oldest clonal seed orchards in the Pacific Northwest.

There are eight blocks in this orchard (Figure 1) each containing clones of ortets from one or more of three different elevations (LOW = less than 600 meters, MID = 600 to 900 meters, HIGH = greater than 900 meters) on the west side of the Cascades in central Oregon. There is virtually no physical separation between blocks or any isolation from the surrounding natural population of Douglas-fir. At the time of this study, ramets had an approximate spacing of 11 m^2, but because of the loss of ramets from graft incompatibility, spacing was not uniform. There were 25 clones representing the low elevational zone, 21 clones from the mid, and 65 clones from the high.

The original placement of ramets in block A (the oldest block) was through a randomized design by R.K. Campbell (Pacific Northwest Forest and Range Experiment Station, Corvallis, OR), which insured that ramets of the same clone were not adjacent and that all clones were equally represented. For all other orchard blocks, there was no systematic design. Ramet placement was at random with the restriction that ramets of the same clone were not placed next to one

another. Some clones were often planted in more than one block, and two to three elevational zones were often represented in the same block. Year(s) in which the clones were initially grafted and the clonal composition of each block are presented in Table 1.

In April 1980, the number of male and female flowers produced by each ramet in block A was estimated according to a visual classification scheme. In addition, every three to four days, this block was surveyed so that the date of female receptivity and pollen shedding of each ramet could be recorded. This survey was continued for 18 days, by which time the flowering of all ramets had begun. Data on flower productivity and phenology of ramets were not recorded in any other block.

In the fall of 1980, cones were collected from upper and lower crown positions of three top-pruned and three non-top-pruned (top-pruned and non-top-pruned will hereafter be referred to as pruned and nonpruned) ramets of six clones (clones 160, 163, 168, 169, 173, and 199), giving a total of 2 (crown positions) x 2 (pruning treatments) x 6 (clones) x 3 (ramets per clone-treatment combination) = 72 separate samples. The cones were sampled by operational harvesting crews which were instructed to collect cones (2-25) from the upper one-third and lower one-third portions of the crown (a visual delineation) of each sampled ramet. Cones and seeds were kept separate by crown position, clone, ramet, and pruning treatment throughout collection, processing, and storage. All seeds were the result of open-pollination.

All pruned ramets were located in block A where all ramets had been pruned to 20 feet in 1968-1969 and again in 1978 to a height of 30 feet. The nonpruned ramets sampled were located in four other blocks (B, C, D, and E, Figure 1) where pruning had not been practiced. Ramets in these blocks were grafted anywhere from one to six years later than those in block A (Table 1). Thus, in addition to the difference in pruning, nonpruned ramets (14 to 19 years from grafting) were, on the average, slightly smaller, narrower crowned, and less crowded than ramets (20 years from grafting) in block A. However, flowering was heavy in all blocks. In fact, the total number of bushels of cones (1,154) was the largest ever recorded at the D.T. Mason Seed Orchard.

The six clones selected for sampling were chosen based on the flowering data collected in block A. Ramets of these clones all flowered within seven days of each other and were intermediate in time of flowering relative to all clones in the orchard block. Thus, clones with unusually early or late flowering periods were avoided. The average flowering date (male and female) in the chosen clones and for block A was April 17. The range in dates among ramets in block A was April 7 to April 25, while the range among ramets of the six clones was April 15 to April 18. The reason for this type of selection was to choose clones which flowered during the heaviest period of flowering and to prevent the selection of clones which were not in synchrony with the majority of clones in the orchard. Individual ramets of each clone were chosen at random from those available with the restriction that they had to be producing large numbers of cones.

II.2. Estimation of proportion of selfed progeny

Estimates of the proportion of viable progeny due to self-fertilization (s) were based on electrophoretic analyses of germinated seeds. Both megagametophyte and embryo tissues of each seed were assayed at 10 allozyme loci using tissue preparation and electrophoretic techniques similar to those described in Conkle et al. (1982), Adams and Joly (1980b) and Neale et al. (1982). All gels were prepared using 12 percent Sigma starch (Sigma Chemical Co., St. Louis, MO).

The 10 loci assayed were chosen because they coded enzymes with clear banding patterns in both seed tissues. These loci were: PGM1 (phosphoglucomutase), LAP1 and LAP2 (leucine aminopeptidase), GOT2 and GOT3 (glutamate-oxaloacetate transaminase), GLYDH (glyceraldehyde-3-phosphate dehydrogenase), CAT (catalase), 6PGD (6-phosphogluconate dehydrogenase), IDH (isocitrate dehydrogenase), and DIA (diaphorase). Descriptions of banding patterns and the Mendelian inheritance of all enzymes except LAP and CAT have been previously reported (El-Kassaby et al. 1982, Neale et al. 1982). The formal genetics of LAP and CAT have been confirmed by W.T. Adams (unpublished data).

Electrophoretic assay of seeds provided two types of genetic data required for estimating s. Segregation of alleles in the mega-gametophytes of each clone was used to infer their genotypes. In a sample of N megagametophytes, the probability of incorrectly inferring

the genotype of a clone at any locus is less than $(1/2)^{N-1}$ (e.g., for a sample of 10 megagametophytes, the probability is less than 0.002). Additionally, by analyzing both seed tissues, the genotypes of the pollen gametes successful in fertilization of viable embryos (called the pollen pool) from each clone can be determined (Müller 1976).

Seeds assayed electrophoretically were first subjected to x-ray analysis to estimate the proportions of filled seed (PF). The original intent was then to assay seeds from two ramets of each of the 24 pruning treatment (A) x clone (B) x crown position (D) (A x B x D) combinations in the study. However, preliminary analysis based on the segregation of alleles in the megagametophytes from each of the original 72 seed collections revealed that three of the nonpruned ramets (two from clone 163, and one from clone 160) included in the sample had been mislabelled in the orchard (i.e., had genotypes which did not match other supposed ramets of the same clone but did match other clones in the orchard). Thus, while seeds for electrophoretic analysis were sampled from two ramets in 22 of the A x B x D combinations, the nonpruned upper and lower crown combinations of clone 163 were represented by seeds from only one ramet. An average of 61 seeds (range 12 to 85) from each A x B x D combination were germinated and assayed electrophoretically (total number 1,464 seeds). For the 22 A x B x D combinations involving two ramets, seeds from each ramet were represented about equally. However, the data from the two ramets of each clone were combined prior to analysis.

Additionally, a concurrent study in our electrophoresis laboratory (W.T. Adams and D.B. Bailey) provided allozyme information on 24 of the 25 clones in block A. Seeds from two ramets of 22 of the clones and one ramet of two of the clones were assayed for the same 10 loci as above; no seeds were available from the one remaining clone in this block. On the average, 9.3 (range 1 to 14) seeds were assayed per ramet, giving a total of 426 seeds in the sample. These samples were used to obtain average estimates of allele frequencies in the outcross pollen pool of block A, and also to obtain an average estimate of the proportion of selfs over the entire block.

Both single- and multiple-locus methods were employed to estimate s. These will be described separately below.

II.2.1. Single locus estimation

Using single locus estimation procedures based on the mixed mating model (Brown and Allard 1970, Clegg et al. 1978), population estimates of proportions of viable outcrossed (t) and selfed (s = 1-t) progeny can be obtained. Modifying the procedure described by Shaw and Allard (1982) to accommodate either two or three alleles at a locus, we estimated t values for nine combinations of crown position and pruning treatment based on the aggregate data of all six clones; and for block A as a whole, utilizing the data for the 24 clones (Table 2).

In the mixed mating model, it is assumed that no selection occurs between germination and the scoring of seed tissue, and each viable

seed is the result of random outcrossing (with probability t) or self-fertilization (with probability s). No other types of related matings are considered in the model. In addition, the model assumes that both t and pollen gametic frequencies are uniform over maternal genotypes. Thus, for each maternal genotypic class, the expected frequency of pollen gametic types in the progeny can be derived (Shaw and Allard 1982). In the simplest case, for example, consider a single marker locus with two alleles, A_1 and A_2, where p and q are the frequencies of A_1 and A_2 in the outcross pollen pool. In the maternal genotypic class A_1A_1, two pollen gametic types in the progeny can be expected: A_1 with probability = s+tp and A_2 with probability = tq. Similar expectations can be derived for the remaining maternal genotypic classes in the diallelic case (i.e., A_1A_2, A_2A_2). For each maternal class, pollen gametic expectations are combined with the observed numbers of pollen gametes for each maternal class, to form a log-likelihood function (see Appendix in Shaw and Allard 1982). Maximum-likelihood estimates of t and p are obtained by solving for the maximum of the joint log-likelihood function over all maternal classes; s (= 1-t) and q (= 1-p) are determined by subtraction. In the triallelic case, three alleles (p, q, r) in the outcross pollen pool are jointly estimated along with t.

In order to be able to estimate t and p for any one locus using the diallelic model, segregating progenies from at least two maternal genotypic classes are required. This is because at least two degrees of freedom are necessary in order to estimate the two mating system parameters (t and p). For example, in the two allele case, consider

the maternal class A_1A_1 once again. In specifying the expectations of pollen gametes A_1 and A_2 for this maternal class, there is only one degree of freedom (for estimation of t and p), because once the expectation of pollen gamete A_1 (s + tp) is specified, the expectation of pollen gamete A_2 is known by subtraction (tq = 1 - (s + tp)). One degree of freedom is insufficient to calculate two parameters. When two or three maternal genotypic classes with segregating progenies are present among the clones, a sufficient number of degrees of freedom (i.e., two or three) are available to estimate both t and p. Because the six clones were monomorphic for 5 of the 10 loci assayed, single locus estimates of t could only be obtained for LAP1, GOT3, GLYDH, CAT, and DIA (Populations A-I, Table 2). However, with the sample of 24 clones in block A (Population J, Table 2), a sufficient number of maternal genotypic classes were available at all loci so that 10 single locus outcrossing estimates were calculated.

The diallelic model was used when there were only two alleles present in the pollen pool at a locus (LAP2, GLYDH, CAT, and DIA) or in the case of GOT3 when 3 alleles were present, but the third allele occurred in low frequency (less than 6 percent). The triallelic model was utilized for loci when three or more pollen alleles were detected (PGM1, LAP1, GOT2, 6PGD, and IDH). In all cases, where combining of alleles was required, data for the most infrequent allelic classes were bulked into one synthetic class utilized in the analysis. It is possible to derive single locus outcrossing models to accomodate more than three alleles; but, computation of estimates becomes

increasingly difficult with each additional allele. Furthermore, adding alleles would not likely lead to significantly increased precision in estimating t, because additional alleles occur at very low frequencies, and thus, would add little information about t.

Homogeneity of single locus t estimates over loci and over different treatment combinations was tested using a chi-square test of homogeneity (Rao 1973, Section 6a):

$$\chi^2 = \sum_{i=1}^{k} [\hat{t}_i - \bar{\hat{t}}]^2 / s^2_{\hat{t}_i} \quad , \quad (k - 1)df$$

$\hat{t}_i$ = single locus outcrossing estimates

k = number of t estimates

$s^2_{\hat{t}_i}$ = sample variance for $\hat{t}_i$

$\bar{\hat{t}}$ = weighted mean of $\hat{t}_i = \sum_{i=1}^{k} [\hat{t}_i / s^2_{\hat{t}_i}] / \sum_{i=1}^{k} [1 / s^2_{\hat{t}_i}]$

II.2.2. Muliple locus estimation

An alternative to the single locus approach is a multilocus estimation procedure. While this method is also based on mixed-mating model assumptions, it differs from single locus estimation in that the pollen gamete of each embryo is assigned to either a detectable outcross class or an ambiguous class, based on the comparison of its multilocus genotype with the genotype of the maternal parent.

The ambiguous class consists of two types of matings: 1) self-fertilizations, and 2) fertilizations by outcross pollen grains which carry alleles identical to the maternal parent over all loci scored. Detectable outcrosses are identified whenever a pollen gamete carries an allele at one or more of the several loci classified, which could not have come from the maternal parent (Shaw et al. 1981). Thus, the expected proportion of detectable outcrosses in the progeny of maternal parent i is $r_i = t_{m_i} G_i$, where t_{m_i} is the proportion of progeny due to outcrossing (multilocus outcrossing rate) and G_i is the conditional probability of detecting an outcross pollen gamete in progeny of maternal parent i, given that an outcross has occurred (detection probability).

Using the maximum likelihood method of Green et al. (1980), population estimates of t_m were calculated for the same set of populations for which single locus estimates were obtained (Table 2). Because the data are combined over many different maternal genotypes, an explicit solution for t_m cannot be derived and $\hat{t}_m$ must be obtained by the numerical solution of the equation:

$$\sum_i [(n_i - O_i)G_i/(1-G_i t_m)] - \sum_i O_i/t_m \simeq 0$$, where O_i is the number of detectable outcrosses in the n_i pollen gametes sampled in progeny of the ith clone.

The detection probability for each maternal genotype (G_i) is the probability of drawing a gamete from the outcross pollen pool with at

least one allele, at one locus, different from those carried by the maternal parent. It was estimated in the following manner:

$G_i = 1 - \prod_{j=1}^{k} f_{ij}$, where f_{ij} = combined frequency of alleles in the outcross pollen pool at locus j that are identical to the alleles at locus j in the ith maternal parent. This is computed over the k loci scored. Estimates of allelic frequencies in the outcross pollen pool used to calculate the f_{ij}'s were those obtained from the single locus estimation procedure described in the previous section. For each population (Table 2), pollen pool allelic frequencies estimated from the same set of data were available for five of the 10 loci. Recall, however, that single locus estimates for the remaining five loci could not be obtained for the six clone samples. Nevertheless, the efficiency of the multilocus estimator would be greatly improved if all 10 loci scored could be utilized in the analysis. To make it possible to take advantage of all 10 loci, estimates of pollen pool frequencies from the total block A sample (J, Table 2) were used for deriving f_{ij}'s for the remaining loci. Estimated pollen pool frequencies were not found to differ appreciably between any of the six clone populations (A-I, Table 2) and block A, for the five loci where estimates could be obtained for all populations. Assuming that this is also the case for the remaining loci, the use of the block A estimates seems warranted. In any case, these are our best estimates of pollen pool allelic frequencies at the five remaining loci.

Finally, the large sample variance of t_m was calculated using the formula given by Green et al. (1980):

$$[\text{var } (t_m)]^{-1} = \sum_i (n_i - O_i)(G_i/(1-G_i t_m)^2 + \sum_i O_i/t_m^2$$

It needs to be recognized that this variance formula assumes that the true G_i values are known (i.e., are not estimates). Thus, $\text{var}(\hat{t}_m)$ must be considered a minimum variance estimate.

Shaw et al. (1981) have also developed a multilocus estimator of t. While the assumptions of their model are the same as the multilocus method used in this study (i.e., the mixed mating model assumptions), their model relies on a method of moments estimator rather than a maximum likelihood procedure. To compare estimates obtained using the multilocus method described in this paper with those obtained using the Shaw et al. method, t was also estimated for populations I and J (Table 2) using the method of moments estimator. The formula of this multilocus estimator (t_{mm}) is:

$$\hat{t}_{mm} = \frac{n}{N(1-\alpha)}, \quad \text{where}$$

n = number of detectable outcrossed progeny in a sample of size N

$$\alpha = \prod_{k=1}^{x} [\sum_{ij} f_k(A_iA_j)\ (p_{ik} + p_{jk}) + \sum_i f_k(A_iA_i)\ (p_{ik})] \quad . \quad . \quad i<j$$

α is the population probability of not identifying an outcross, given that an outcross has occurred. In the latter equation, $f_k(A_iA_i)$ and $f_k(A_iA_j)$ are the estimated frequencies of genotypes

A_iA_i and A_iA_j at the kth locus, weighted by the number of progeny each genotype contributes, for x loci. p_{ik} and p_{jk} are pollen allelic frequencies at the kth locus. The principal difference between the Shaw et al. (1981) and Green et al. (1980) methods is that a detection probability is determined for the entire population in the method of moments estimator, while detection probabilities are calculated separately for each clone in the maximum likelihood model.

Multilocus procedures were also utilized to estimate t_m for each of the six clones individually. Although sample sizes were limited, estimates were calculated separately for each crown position of pruned and nonpruned ramets. No estimate was computed for the lower crown of nonpruned ramets of clone 163 where only 12 observations were available; for all other combinations, the observed number of pollen gametes per sample ranged from 46 to 85.

The maximum likelihood estimator for the proportion of progeny of a single clone due to random outcrossing ($t_{m_i} = 1-s_{m_i}$) is given by the formula:

$$\hat{t}_{m_i} = \frac{O_i}{n_i \hat{G}_i} = \frac{\hat{r}_i}{\hat{G}_i} \text{ , where } \hat{r}_i = \frac{O_i}{n_i}$$

$\hat{G}_i$ = detection probability for the ith clone

The G_i's utilized for each clone were those derived for the population estimates of t_m and were those appropriate for each particular crown position and pruning treatment combination (e.g., G_i's derived from population D (Table 2) were used in estimating t_{m_i}'s for upper crowns of nonpruned ramets).

Since $\hat{t}_{m_i}$ is a function of two variables, $\hat{r}_i$ and $\hat{G}_i$, an approximation of the variance of $\hat{t}_{m_i}$ is:

$$\text{var } \hat{t}_{m_i} = \left[\frac{\partial \hat{t}_i}{\partial \hat{r}_i}\right]^2 \text{var } \hat{r}_i + \left[\frac{\partial \hat{t}_i}{\partial \hat{G}_i}\right]^2 \text{var } \hat{G}_i + 2\left[\frac{\partial \hat{t}_i}{\partial \hat{r}_i}\right]\left[\frac{\partial \hat{t}_i}{\partial \hat{G}_i}\right] \text{cov } (\hat{r}_i, \hat{G}_i)$$

and since $\frac{\partial \hat{t}_i}{\partial \hat{r}_i} = \frac{1}{\hat{G}_i}$, $\frac{\partial \hat{t}_i}{\partial \hat{G}_i} = -\frac{\hat{r}_i}{\hat{G}_i^2}$, then

$$\text{var } \hat{t}_{m_i} = \left[\frac{1}{\hat{G}_i^2}\right] \text{var } \hat{r}_i + \left[\frac{\hat{r}_i^2}{\hat{G}_i^4}\right] \text{var } \hat{G}_i - \left[\frac{2\hat{r}_i}{\hat{G}_i^3}\right] \text{cov } (\hat{r}_i, \hat{G}_i).$$

Large sample approximations for the variances of $\hat{r}_i$ and $\hat{G}_i$ can be calculated fairly readily,

$$\text{var } \hat{r}_i = \frac{\hat{r}_i(1-\hat{r}_i)}{n_i} \quad ; \quad \text{var } G_i = \sum_{j=1}^{k} (\text{var } f_{ij}) \left(\prod_{j=1}^{k} f_{ij}^2\right), \text{ where}$$

k = number of loci and f_{ij} is as defined previously.

Nevertheless, cov $(\hat{r}_i, \hat{G}_i)$ is extremely complicated and we have not been able to derive an expression for it. If r_i and G_i are estimated from independent samples, than cov $(\hat{r}_i, \hat{G}_i) = 0$ and the calculation of var $\hat{t}_{m_i}$ is simplified. Experimentally, $\hat{r}_i$ and $\hat{G}_i$ above can be made independent by calculating the G_i for each clone with progeny data from only the other clones, i.e., in estimating G_i, the outcross pollen gametic frequencies are generated from a data set

excluding the progeny of the ith clone (independent data set). However, this requires calculating a separate set of pollen pool allele frequencies for each clone.

On the other hand, if the n_i progeny from the ith clone are included in the progeny data used to estimate pollen pool allele frequencies, cov $(\hat{r}_i, \hat{G}_i)$ would be expected to be small if n_i is small in relation to the size ($N = \Sigma n_i$) of the total (or combined) data set. If the cov $(\hat{r}_i, \hat{G}_i)$ is indeed negligible, then only one set of pollen pool frequencies needs to be calculated for all parent trees, reducing greatly the computations required.

To determine the consequences of ignoring cov $(\hat{r}_i, \hat{G}_i)$ when $\hat{r}_i$ and $\hat{G}_i$ do not come from independent samples, estimates of G_i, t_{m_i} and var t_{m_i} calculated with outcross pollen pool frequencies from combined data sets (and assuming cov $(\hat{r}_i, \hat{G}_i) = 0$) were compared to those obtained when independent data sets were utilized. Upper crown estimates based on independent and combined data sets, for pruned ramets of the six clones, are shown in Table 3. In this case, the sample size (N) for the combined data set was 422, and the contribution of each clone's progeny to N ranged from 15 (clone 163) to 20 percent (clone 168). Clonal values of $\hat{G}_i$, $\hat{t}_{m_i}$ and $s^2_{\hat{t}_{m_i}}$ based on pollen pool allelic frequencies derived from independent or combined data sets were virtually identical. Therefore, for ease of calculation, combined data sets were used to derive G_i for all t_{m_i} estimates, and cov (r_i, G_i) was ignored in calculating var t_{m_i}.

Whether multilocus outcrossing rates are based on maximum

likelihood or method of moments estimation, there are three basic advantages of the multilocus technique compared to single locus procedures. First, the probability of directly detecting an outcross event increases as more marker loci are considered. Second, the multilocus procedure is less sensitive to violations of mixed mating model assumptions as more outcrosses are detected by direct observation (Shaw et al. 1981). Finally, while the single locus method can only estimate the mean outcrossing rate of a number of clones, both the multilocus maximum likelihood and method of moments procedures can be used to estimate outcrossing rates for individual clones.

II.3. Estimation of proportion of filled seeds

Proportions of filled seeds (PF) for the 72 samples (2 crown positions x 2 pruning treatments x 6 clones x 3 ramets per clone-treatment combination) were determined by scoring x-ray photographs of seed lots. Only apparent full-sized ("round") seeds, as opposed to "flat" seeds (see Sorensen 1971), were x-rayed. Seeds with developed megagametophytes and embryos were counted as filled. Seeds with insect larvae were assumed to be previously filled and were tallied as such. Any biases attributed to scoring this way were probably very small, since the total percentage of seeds with insect larvae was less than 0.7 percent. The average number of seeds x-rayed in each sample was 198 (range 42 to 275). The total number of seeds examined was 14,274.

The data were analyzed in two ways: 1) by analysis of variance of filled seed proportions and 2) by chi-square heterogeneity tests. Analysis of variance of $\hat{PF}$ values was according to a split-split-plot design, where pruning treatments were considered as main plots, clones as sub-plots, and crown positions as sub-sub-plots. The experimental design differed somewhat from the normal split-split-plot design however, because 1) ramets were nested subsamples within clone-treatment combinations and 2) main plots (pruned versus nonpruned) were not replicated. Clones and ramets were treated as random effects, while crown positions (upper versus lower) and pruning treatments (pruned versus nonpruned) were fixed. The analysis of variance had the form shown in Table 4.

Recall that the isozyme genotypes of two nonpruned ramets of clone 163 and one nonpruned ramet of clone 160 were not in agreement with their respective clonal isozyme genotypes. $\hat{PF}$ values for the upper and lower crown samples taken within each of these three ramets (a total of 2 x 3 = 6 samples) were discarded and treated as missing data. Because of the low number (6) of missing plots and the relative complexity of the experimental design, it was decided to use an approximate procedure to replace the missing plots in the analysis of variance. For clone 163, the upper and lower crown $\hat{PF}$ values for the two missing ramets were replaced with the corresponding filled seed proportions from the one remaining nonpruned ramet of this clone. For clone 160, $\hat{PF}$ values for the one missing ramet were replaced with the mean corresponding $\hat{PF}$ values of the remaining two nonpruned ramets. Analysis of variance was then carried out in the usual

manner, with the exception that the appropriate error degrees of freedom were reduced to account for the loss of information (i.e., substitution of missing values), and error mean squares were calculated using the reduced degrees of freedom. Therefore, all mean squares and F-ratios reported are approximate. Analyses of variance were carried out with both $\hat{PF}$ and an arcsine transformation (Neter and Wasserman 1974, Section 15.5) of $\hat{PF}$ values. Since the results of these two analyses were essentially the same, only the analysis of variance of the nontransformed data is reported.

A more detailed, but also more cumbersome analysis of the PF data was carried out by conducting a large number of heterogeneity chi-square tests. Tests of treatment differences are more precise using chi-square because it takes advantage of the large sample sizes in the proportion data; however, interaction effects are easier to test and interpret with an analysis of variance. The formula for chi-square was from Snedecor and Cochran (1967, Section 9.8). Heterogeneity of $\hat{PF}$ values among clones was tested for each crown position and pruning treatment combination. Furthermore, heterogeneity chi-squares were calculated for testing the average effects of crown position and pruning treatment, when data from all clones were combined.

II.4. Estimation of cone contribution from different crown levels

In order to relate upper and lower crown estimates of s and PF to the total seed crop of a clone, proportions of the seed crop due to cones from different crown levels must be known. To determine

approximately the percent contribution that upper and lower cones make to the total cone harvest of a clone, and to learn additionally whether these percentages vary among clones, additional ramets were surveyed in the fall of 1982. This year was an average flowering year for the orchard; a total of approximately 500 bushels of cones was collected in all blocks (personal communication, Howard Dew - seed orchard manager), which is close to the mean number of bushels (about 440) which has been collected in this orchard once every two years, since 1971. One cone-producing ramet from each of six different clones (clones 167, 172, 173, 208, 209, and 347) was selected at random in block B (see Figure 1). All cones in each ramet were picked and the number of cones in each of three crown positions (upper-third, mid-third, and lower-third) was counted. Crown positions were visually stratified. Because the variances of number of cones per ramet were significantly heterogeneous over crown positions ($P < 0.01$, Steel and Torrie 1980, Section 20.3), all cone numbers were transformed to $\log_{10}$, and an analysis of variance was performed on the transformed cone numbers. Individual ramets (i.e., clones) were treated as random effects and crown positions were fixed. The analysis of variance had the form shown in Table 5.

II.5. Correlations

If low filled seed yields are largely the result of high levels of self-fertilization and/or low self-fertility, strong correlations between PF and t would be expected. Clonal means were used to

calculate the correlations of $\widehat{PF}$ with $\hat{t}_{m_i}$ for each crown position (data for pruned and nonpruned ramets were combined).

The degree to which clones outcross (i.e., the level of t, and indirectly PF) might be influenced by the number of their ramets (NFR) which are flowering in the orchard or their flower productivity (FLP). For example, one might expect that if the NFR (or FLP) value for a particular clone was large, more "self pollen" would be available to its ramets, and the proportion of viable selfs produced by this clone might be relatively high. Utilizing the flowering information collected in block A, the various correlations between clonal means of $\widehat{PF}$ and $\hat{t}_{m_i}$ with NFR and $\widehat{FLP}$ (both male and female) were also calculated.

The methods described in Steel and Torrie (1980, Section 11.4) were used to test whether the estimated correlation coefficients were significantly different from 0.0.

II.6. Inbreeding depression in productivity

While estimates of the proportion of viable selfs in the progeny of orchard clones is useful information for seed orchard managers, it would also be of interest to speculate to what degree such levels of selfing might affect the productivity of Douglas-fir plantations regenerated with seed orchard seed. Sorensen and Miles (1982) have developed a method for calculating losses in the productivity of wind-pollinated Douglas-fir plantations at age 10, based on observed inbreeding depressions in survival and height and on expected

frequencies of self-seedlings in the progeny of wind-pollinated populations. Assumptions of the method include:

(a) no culling in the nursery

(b) inbreeding depression in height at 10 years = 29% (height depression was actually measured in the study by Sorensen and Miles)

(c) inbreeding depression in diameter (not measured) equal to that in height

(d) tree volume = volume of a cone = $\frac{\pi d^2 h}{12}$, where d and h are the average diameter and height of cross-pollinated plants

(e) 17% of selfed plants do not survive in the first two years after field planting (actually measured)

(f) productivity of a plantation without selfed plants is set equal to 1.0

The productivity of a plantation at age 10 established from wind-pollinated seedlings, then, is given by the equation:

productivity =

$$(1.00)(t) + (0.00)(0.17)(s) + \left[\frac{\pi(.71d)^2(.71h)/12}{\pi d^2 h/12} \right] (0.83)(s)$$

t = proportion of viable outcrossed progeny

s = 1 - t = proportion of viable selfed progeny

$\pi(.71d)^2(.71h)/12$ = average volume of a selfed tree

.83 = proportion of selfed plants surviving in the first two years after planting (1 - .17).

The proportions of viable self-fertilized progeny ($\hat{s}_m = 1 - \hat{t}_m$) obtained 1) by calculating the average of multilocus outcrossing

rates among the six clones, and 2) from the population maximum likelihood estimator for block A clones, were used to get a general estimate of the effect that inbreeding depression would have on the productivity of seedlings from the seed orchard. Productivity indices were calculated for the upper, lower, and combined crown positions of the six clones, and for 24 clones in block A.

III. RESULTS

III.1. Single locus estimates of the proportion of progeny due to outcrossing (t)

Five single locus estimates of t ($\hat{t}$) for each of the nine progeny populations of the six clones intensively sampled in this study (A-I, Table 2) and for block A (J, Table 2) are shown in Table 6. The overall population estimate for the six clones ($\hat{t}$ = 0.958) and the population estimate for block A ($\hat{t}$ = 0.960) both indicated that on the average, a high degree of outcrossing is the rule in Douglas-fir seed orchards. However, there was considerable heterogeneity in estimates of t over loci, regardless of the population sample. For example, estimates of t for pruned ramets (PR) of the six clones ranged from 0.682 (CAT) to 1.107 (DIA) among the five loci available for analysis. Similarly, among the ten loci for which it was possible to estimate t in block A, $\hat{t}$ ranged from 0.764 (GLYDH) to 1.046 (PGM1). In fact, in seven of the 10 population samples, heterogeneity chi-square values were significant at at least the five percent level (Table 6).

Heterogeneity among single locus estimates of t has been found previously in Douglas-fir (Shaw and Allard 1982) and other plant species (Allard et al. 1977, Clegg 1980, Moran and Brown 1980). It may reflect the large variances that are common with the estimation of single locus t values or violations of the mixed mating model assumptions (Shaw et al. 1981). Shaw (1980) simulated data which

obeyed all assumptions of the model and still found heterogeneity in t values, suggesting that random effects may contribute to the single locus variability which has been observed in so many studies.

Because of the large heterogeneity of t estimates over loci, no attempt was made to test for differences in t due to crown position or pruning level.

III.2. Multiple locus estimates of the proportion of progeny due to outcrossing (t_m)

The outcross pollen pool allele frequencies determined from the single locus estimation procedure are shown in Table 7. Over all four progeny populations of the six clones (PR-U, PR-L, NP-U, NP-L), estimates of allelic frequencies in the outcross pollen pool were homogeneous at the five loci for which estimates were obtainable. Furthermore, when the data for the six clones were combined over pruning treatments and crown positions (overall) and allelic frequencies compared to the frequencies obtained based on 24 clones (block A), outcross pollen allelic frequencies were found to be homogeneous at all loci except CAT. In fact, estimated outcross pollen allelic frequencies were identical at GLYDH and DIA between these two groups of data. Thus, gametic frequencies at the five loci which could not be calculated directly for the six clones (PGM1, LAP2, GOT2, 6PGD, and IDH), but were estimated from the 24 clone information in block A, were probably valid approximations of allelic frequencies in the

outcross pollen pool of the six clones. These frequencies are footnoted in Table 7.

III.2.1. Population estimates

Population multilocus outcrossing estimates ($\hat{t}_m$) over the six clones for the various crown position and pruning treatment combinations, and for 24 clones are shown in Table 8. Population estimates assume that t is relatively uniform over clones. However, single clone estimates of t ($\hat{t}_{m_i}$) were significantly heterogenous for all progeny samples (Table 8). Any bias due to heterogeneity of t values is compounded because only six genotypes were represented in the samples. Clones with $\hat{t}_{m_i}$ values closer to 1.0 have lower variances and thus data from these clones provided more of the information for estimating the population t ($\hat{t}_m$). This resulted in most of the population estimates being higher than unweighted mean estimates from the six clones. In eight out of nine comparisons, population estimates were slightly larger than unweighted estimates (Table 8). In the rest of the paper, unweighted means of six clones ($\bar{\hat{t}}_{m_i}$) will be used for comparing mean effects of pruning treatments and crown position of cones.

For the progeny of the six clones as a group, a significantly ($P < 0.05$) larger proportion of random outcrosses was found in the upper crowns of both pruned ($\bar{\hat{t}}_{m_i} = 0.943$) and nonpruned ($\bar{\hat{t}}_{m_i} = 0.940$) ramets than in the lower crowns (PR-L: $\bar{\hat{t}}_{m_i} = 0.815$, NP-L: $\bar{\hat{t}}_{m_i} = 0.827$;

Tables 8 and 9). On the average, 16.7 percent more outcrosses were observed in the upper ($\bar{\hat{t}}_{m_i}$ = 0.945) than in the lower ($\bar{\hat{t}}_{m_i}$ = 0.810) crown. In addition, there was no significant ($P > 0.05$) difference in $\bar{\hat{t}}_m$ between pruned and nonpruned ramets in both upper crowns (PR-U: $\bar{\hat{t}}_{m_i}$ = 0.943, NP-U: $\bar{\hat{t}}_{m_i}$ = 0.940) and lower crowns (PR-L: $\bar{\hat{t}}_{m_i}$ = 0.815, NP-L: $\bar{\hat{t}}_{m_i}$ = 0.827), and when crown positions were combined (PR: $\bar{\hat{t}}_{m_i}$ = 0.884, NP: $\bar{\hat{t}}_{m_i}$ = 0.908) (Tables 8 and 9). The overall population $\bar{\hat{t}}_{m_i}$ value for the six clones (0.894) was very similar to the population estimate for 24 clones in block A (0.935) (χ^2_1 = 1.89, $P > 0.05$). Estimates of t derived by the Shaw et al. (1981) method of moments estimator (t_{mm}) were nearly identical to the maximum likelihood population estimates (t_m, Table 8). For the six clones, the overall estimate of t_{mm} was 0.913 ($\hat{t}_m$ = 0.915, χ^2_1 = 0.01, $P > 0.05$), and for the 24 clones in block A, the t_{mm} value was 0.921 ($\hat{t}_m$ = 0.935, χ^2_1 = 0.13, $P > 0.05$).

III.2.2. Single clone estimates

Outcrossing estimates were significantly heterogeneous over clones for all progeny populations (Table 8). However, the range in $\hat{t}_{m_i}$ was always greater in the lower crown (PR-L: 0.457 to 1.105, NP-L: 0.484 to 1.079, L: 0.525 to 1.009) than in corresponding upper crown samples (PR-U: 0.817 to 1.132, NP-U: 0.812 to 1.080, U: 0.837 to 1.029).

On the average, estimates of outcrossing were lower in the lower crown. But, most of the mean effect was due to large differences

($P < 0.01$) in $\hat{t}_{m_i}$ with respect to crown position in clones 160 ($\chi^2_1 = 9.80$) and 163 ($\chi^2_1 = 27.67$). These were no significant differences between upper and lower crown estimates of t_{m_i} for the remaining clones. In addition, there was no consistent pattern with pruned and nonpruned clonal $\hat{t}_{m_i}$ values. Values were significantly ($P < 0.01$) lower for pruned ramets of clones 163 ($\chi^2_1 = 26.19$) and 169 ($\chi^2_1 = 7.87$), but there was a larger proportion of outcross progeny estimated for nonpruned ramets of clone 173 ($\chi^2_1 = 7.28$, $P < 0.01$). For the remaining clones (160, 168, and 199) there were no significant differences between pruned and nonpruned estimates of $\hat{t}_{m_i}$. The inconsistency of the relationship between clonal values was reflected by the comparison of unweighted population means, i.e., $\bar{\hat{t}}_{m_i}$'s were nearly identical in pruned and nonpruned treatments (Table 9).

III.3. Proportion of filled seeds estimates

The analysis of variance of untransformed $\widehat{PF}$ values is presented in Table 10. Approximate F-tests revealed that the mean squares for all interaction effects were nonsignificant ($P > 0.05$). Mean squares for both clones and crown position however, were significant, indicating that genotype and crown position are both important factors affecting filled seed proportions in orchard clones. Mean proportions of filled seeds ranged over two-fold among clones (0.301 (clone 160) to 0.643 (clone 168)), and consistent with earlier studies in other species (Franklin 1971b, Hadders 1971, Squillace and Goddard 1982), PF was found to be considerably higher in upper crown cones

($\widehat{PF}$ = 0.512) than in cones from the lower crown ($\widehat{PF}$ = 0.381; Table 11). Since the clone x position mean square was nonsignificant, crown position differences would be expected to be relatively consistent over clones. Only in one clone (173) was the mean $\widehat{PF}$ value smaller in the upper than the lower crown (Table 11), and in this case, the difference was very small (0.555 versus 0.573). Mean percentage of filled seeds differed very little between pruned ($\widehat{PF}$ = 0.432) and nonpruned ramets ($\widehat{PF}$ = 0.461; Table 11), and the difference was not significant (Table 10).

An analysis of heterogeneity chi-square values for the same set of data leads to similar results as found with the analysis of variance. For example, significant heterogeneity of filled seed proportions was found among clones for all comparisons (i.e., whether data were combined over crown levels and pruning treatment or whether clonal differences were examined separately for each crown level x pruning treatment combination; Table 12). In addition, when averaged over all clones, cones from the upper crown had significantly more filled seeds in both pruned and nonpruned ramets (Table 13). The consistency of this relationship over clones is supported by the observation that when data from pruning treatments were combined, differences in five out of six clonal comparisons were significant, with the upper crown samples having a greater PF than the lower. Only for clone 173 did the lower crown have a higher proportion, but the difference was not significant (Table 13).

In the chi-square analysis, the slightly greater mean proportion of filled seeds in nonpruned versus pruned ramets was found to be

significant (χ^2_1 = 25.31, (P < 0.01) Table 13). Recall, in the analysis of variance the mean square for this comparison was not significant. This result, however, seems to be mostly due to the significant difference between pruned and nonpruned ramets (χ^2_1 = 46.76 (P < 0.01)) in the lower crown; the difference in filled seed percentages in the upper crown was not significant (Table 13).

III.4. Cone contribution from different crown levels

Out of a total of 1,812 cones tallied over the six clones sampled in 1982, 887 cones (49 percent) were observed for the upper crown, 754 cones (42 percent) for the mid-crown region, and 171 cones (9 percent) for the lower crown (Table 14). Analysis of variance of cone numbers ($\log_{10}$) revealed no significant difference in cone number among clones, but there was a significant (P < 0.05) difference among crown positions (Table 15). When transformed crown position means were compared pairwise by Tukey's test (Steel and Torrie 1980, Section 8.6) significant differences were found between mean cone numbers in the upper and lower crown (P < 0.05) and middle versus lower crown (P < 0.05); but no difference was found between the upper and middle crown means. The mean number of cones retransformed from $\log_{10}$ was 91.20, 77.62, and 19.95 for the upper, middle, and lower crown regions.

Although this was a very small sampling of orchard ramets, the results are consistent with previous observations of other monoecious species (Nienstaedt 1981, Squillace and Goddard 1982) in which lower

parts of the crown contribute relatively few cones. In Picea glauca, this relationship appears to be true especially for top-pruned seed orchard grafts (Nienstaedt 1981). It was found that on top-pruned trees, the most productive whorl was the first one below the top-pruning treatment; while for untreated grafts, the fourth whorl below the terminal bud was the most productive.

III.5. Correlations

No significant correlations (4 d.f., $P > 0.05$) could be found between mean filled seed proportions ($\widehat{PF}$) and outcrossing rates ($\hat{t}_{m_i}$) per clone for the six intensively sampled clones (data from pruned and nonpruned ramets combined) whether crown positions were analyzed separately (upper crown correlation: $r = -0.13$; lower crown correlation: $r = 0.57$) or combined ($r = 0.33$).

Number of ramets which were flowering in the orchard for the six clones and estimates of total number of male and female flowers produced by these ramets in block A are given in Table 16. These data made it possible to investigate any associations between mean $\widehat{PF}$ or $\hat{t}_{m_i}$ in the pruned clones with their number of flowering ramets (NFR) or flowering productivity (FLP). Mean $\widehat{PF}$ or $\hat{t}_{m_i}$ values per clone did not correlate significantly with NFR or $\widehat{FLP}$ (male or female), whether data for clonal means of $\widehat{PF}$ and $\hat{t}_{m_i}$ were combined over crown positions or whether crown positions were analyzed separately. With only six data points, however, it would have been possible to detect only very strong correlations. Although nonsignificant, correlations

between $\widehat{PF}$ in the upper crown with 1) number of flowering ramets ($r = -0.69$, $0.1 < P < 0.2$), and 2) amount of male flowers among ramets in block A ($r = -0.74$, $.05 < P < 0.1$) were consistent with expectations. Lower outcrossing estimates and more empty seeds were expected for clones with large numbers of flowering ramets or large estimates of male flowering productivity.

III.6. Inbreeding depression in productivity

The estimated relative productivity values (PV) of Douglas-fir plantations (age 10) established with wind-pollinated progenies from the upper, lower, and total crown of six clones and from 24 clones in block A, are shown in Table 17. For a plantation established with progenies from the 24 clones, $\widehat{PV}$ was 0.95, which means that after 10 years, the estimated productivity loss due to natural self-fertilizations is five percent. For the six clones, an upper crown cone collection would result in a productivity increase of 1) 10 percent relative to a lower crown collection, and 2) three percent relative to a total crown collection. Recall that the two crown positions represented the upper- and lower-thirds; the total crown PV estimate was derived by estimating an outcrossing rate for a total crown collection:

$$\text{total crown } \hat{t} = \frac{(0.49)\ 0.945 + (0.42)\ 0.878 + (0.09)\ 0.810}{0.49 + 0.42 + 0.09} = 0.905,$$

where

0.49, 0.42, 0.09 equal the estimated mean proportionate contributions of cones from the upper, mid, and lower crowns, respectively, to the total cone harvest (Table 14), and 0.945 and 0.810 are the t_{m_i} estimates for the upper and lower crowns (Table 8). The mean mid-crown t_{m_i} for the six clones (0.878) was estimated by averaging the upper and lower crown values.

Values of $\bar{\hat{t}}_{m_i}$ (0.905) and $\bar{\hat{s}}_{m_i}$ ($1-\bar{\hat{t}}_{m_i}$ = 0.095) were then used in the Sorensen and Miles (1982) productivity formula to estimate PV for a total crown collection. Total crown estimates assume an equal number of seeds for all crown positions. Table 18 shows weighted total crown estimates for PF, s, and PV.

IV. DISCUSSION

The results of this study indicate that on the average, only a small proportion of the viable progeny produced in Douglas-fir seed orchards is due to selfing. The overall frequency of viable selfs ($1-\bar{\hat{t}}_{m_i}$) in the progeny of the six clones as a group was 0.10 (Table 18), and 0.07 for the block A clones. Not only are these estimates consistent with what Shaw and Allard (1982) found for a Douglas-fir seed orchard ($\hat{s}$ = 0.09), but they are in close agreement with selfing rates found in natural populations of Douglas-fir (range 7 to 14 percent). Because estimates varied only slightly between the eight populations they surveyed, Shaw and Allard (1982) concluded that 90% outcrossing and 10% selfed progeny may be typical of coastal Douglas-fir populations in the Pacific Northwest. The implication for orchard management is that under present orchard designs, i.e., where an effort has been made to keep ramets of the same clone separated, the frequency of viable self seeds is no greater than that found in natural stands.

Although the average frequency of selfs for the orchard clones indicated a high level of outcrossing, there was considerable variability in both seed set (range $\widehat{PF}$ = 0.285 to 0.643) and outcrossing rate (range $\hat{t}_{m_i}$ = 0.707 to 1.014) among the six clones intensively sampled. When considering the fact that the six clones were chosen based on similarity in flowering periods, the range in seed set or outcrossing rate among these clones is probably a conservative estimate of the true variability among all orchard clones.

Evidence from another Douglas-fir seed orchard study (Shaw and Allard 1982) seems to substantiate this idea. Among nine families (families which were identified to be the most "highly selfed" and most "highly outcrossed", based on a previous survey of families in the orchard), outcrossing values were estimated to range from 0.56 to 1.13. This large range may have been due to a variety of factors such as 1) clones which did not flower at the same time (i.e., early or late) with the majority of clones, 2) variable self-fertility among clones, or 3) age and design of orchard.

Clone 160 had the lowest mean outcrossing rate ($\hat{t}_{m_i} = 0.707$) and the lowest seed set for upper cones ($\widehat{PF} = 0.335$). If certain clones, like 160, are consistently poor performers in these two characteristics, then perhaps they should be rogued from the orchard or not included in seed collections.

16.7 percent more outcrosses were observed in seeds from the upper crown compared to the lower crown for the six clones as a group. This difference with respect to crown position appears to be greater than that found by Shaw and Allard (1982) for Douglas-fir, i.e., 8 percent for natural populations and 3 percent for a seed orchard. However, recall that for four of the six clones, there was no significant difference between upper and lower crown estimates of t_{m_i}. On the average, differences in outcrossing estimates between upper and lower crowns in Douglas-fir seed orchards may not be that large.

The trade-offs between upper and lower crown cone collections can be summarized in Table 18. Because of low seed yields and higher

proportions of selfed progeny in cones from lower crowns, Douglas-fir seed orchard managers may consider not collecting lower crown cones. If lower cones were left on the tree, there would be a loss of approximately nine percent of the total cone crop, but savings would be made on overall collection time. Nine percent of the total cone production from lower crowns represents about seven percent

$$\left[\frac{(0.38)(0.09)}{(0.51)(0.49) + (0.45)(0.42) + (0.38)(0.09)} = 0.07 \right]$$

of the total filled seed yield. However, the proportion of filled seeds in an upper and mid crown collection would be approximately 48 percent

$$\left[\frac{(0.51)(0.49) + (0.45)(0.42)}{0.49 + 0.42} = 0.48 \right],$$

and this is an increase of two percent over a collection which included the lower cones (total crown $\hat{PF} = 0.47$). Thus, seed processing costs might be less expensive because there would be relatively less empty seeds in an upper and mid crown collection. In addition, the proportion of viable selfs would average nine percent

$$\left[\frac{(0.06)(0.49) + (0.12)(0.42)}{0.49 + 0.42} = 0.09 \right],$$

and total productivity of plantations at age 10 established from wind-pollinated seeds from upper and mid crowns would be 0.94. The former figure represents a 10 percent decrease over a total crown collection, while the latter is a one percent increase.

One option which could be used to improve the total seed yield could be the utilization of supplemental mass pollination. This could increase both the quantity and genetic quality of the total crop, especially in lower crown cones, but the relatively small proportion of cones in the lower crown might still not justify their collection. Squillace and Goddard (1982) suggested that perhaps the greatest benefits of mass pollination would occur if lower crowns were given the most attention. However, it must be considered again that lower cones contribute only a small proportion to the total cone yield, and may not warrant the costs of a lower crown mass pollination procedure.

Crown position effects on the proportion of viable selfed progeny also suggest that when using seeds for experimental purposes, sampling should occur from a specified crown position. For example, in open-pollinated progeny tests, it is usually assumed that all individuals within families are half-sibs. But, inbreeding (due to selfing) will contribute to a greater correlation among individuals within families than normally assumed for half-sibs (i.e., greater than 0.25) and heritability will likely be overestimated (Squillace 1974). Thus, some of the biases attributed to natural inbreeding could be avoided by collecting seed only from the upper crown (Franklin 1971b). On the other hand, if one wishes to locate trees heterozygous for recessive mutant alleles, wind-pollinated seeds from lower crown collections would be more useful than those from the upper crown (Franklin 1971b). Because more selfs are produced in the

lower crown, the chance of recessive marker genes being expressed (i.e., occurring in the homozygous state) is greater.

There was little evidence that pruning affects t. Mean estimates of t_{m_i} for pruned and nonpruned ramets were not significantly different (Table 9). In addition, when $\hat{t}_{m_i}$ values for pruned and nonpruned ramets of individual clones were compared, no consistent relationship was found between pruning treatment and level of outcrossing.

Likewise, pruning appeared to have only a minor impact on seed set. Pruned ramets, on the average, had slightly greater mean proportions of empty seeds (6 percent) and this difference was significant when tested by the heterogeneity chi-square analysis (Table 13). Nevertheless, much of the difference appears to be due to the relatively strong mean difference between pruned and nonpruned ramets in the lower crown (Table 13). There was virtually no mean difference between filled seed percentages in the upper crown, and since most cones in Douglas fir seed orchard clones are found in the upper crown regions, pruning probably reduces filled seed yield in the total crop of a tree only to a minor extent.

Finally, while crown position differences in seed set were consistent with the spatial arrangement of strobili in Douglas-fir (i.e., greater probability of selfing in the lower crown), there was no significant correlation over clones between seed set and outcrossing rate, as one might expect. That is, although clone 160 had both a low seed set and the lowest outcrossing rate, a relationship between the two traits was not found. This implies that

while some of the empty seeds may be due to self pollination, other elements probably also have a strong influence on seed yield. One such element could be pollen availability (Shaw 1980). In block A, the relatively high, but nonsignificant correlations between filled seed proportions in the upper crown with 1) the number of flowering ramets per clone ($r = -0.69$) and 2) the amount of male flowers per clone ($r = -0.74$) were consistent with expectations. Hadders (1971) discovered a significant correlation between the intensity of male flowering and the percentage of empty seeds in the lower crown cones of a Pinus sylvestris seed orchard. Whereas his correlations were based on 34 degrees of freedom (36 grafts minus 2), in our investigation, the degrees of freedom for r were only 4 (6 clones minus 2), and this made it difficult to detect a significant relationship. Nutritional deficiencies related to crown level (Hsin and Daniels, cited in Shaw 1980) may influence seed set in Douglas-fir, and could contribute to the lack of significant relationships ($\widehat{PF}$ with $\hat{t}_{m_i}$, $\widehat{PF}$ or $\hat{t}_{m_i}$ with NFR and $\widehat{FLP}$) between the various parameters investigated. Variability in self-fertility among clones could also be instrumental in confounding these relationships. Highly self-fertile clones, for example, might produce both filled seeds and viable selfs in substantial amounts. Clonal variability in self-fertility is perhaps one of the most likely explanations for the nonsignificant correlations, especially in light of work by Sorensen (1971). He showed that there were large differences in self-fertility (range 0.1 to 45 percent) among 35 Douglas-fir trees.

Selfing in seed orchards has been of considerable interest because of the potential for self-fertilization frequency to be higher than that found in natural populations, and because of the depression in economic traits that usually accompanies inbreeding in conifers. There was no evidence, though, that selfing rates were, on the average, any different for a Douglas-fir seed orchard than rates found in natural stands. In addition, no difference was found between pruned and nonpruned ramets, and there was little evidence that pruning had any effect on seed set. Crown position effects have previously been verified in other studies. However, these questions still remain: 1) Is there a relationship between the severity of top-pruning and the frequency of viable selfs? 2) Does the considerable heterogeneity among clones in seed set or outcrossing rate hold true year after year? 3) Can clones be identified which are consistently poor seed producers and low outcrossers at each cone harvest? Answers to questions such as these would be useful in managing or designing seed orchards more efficiently.

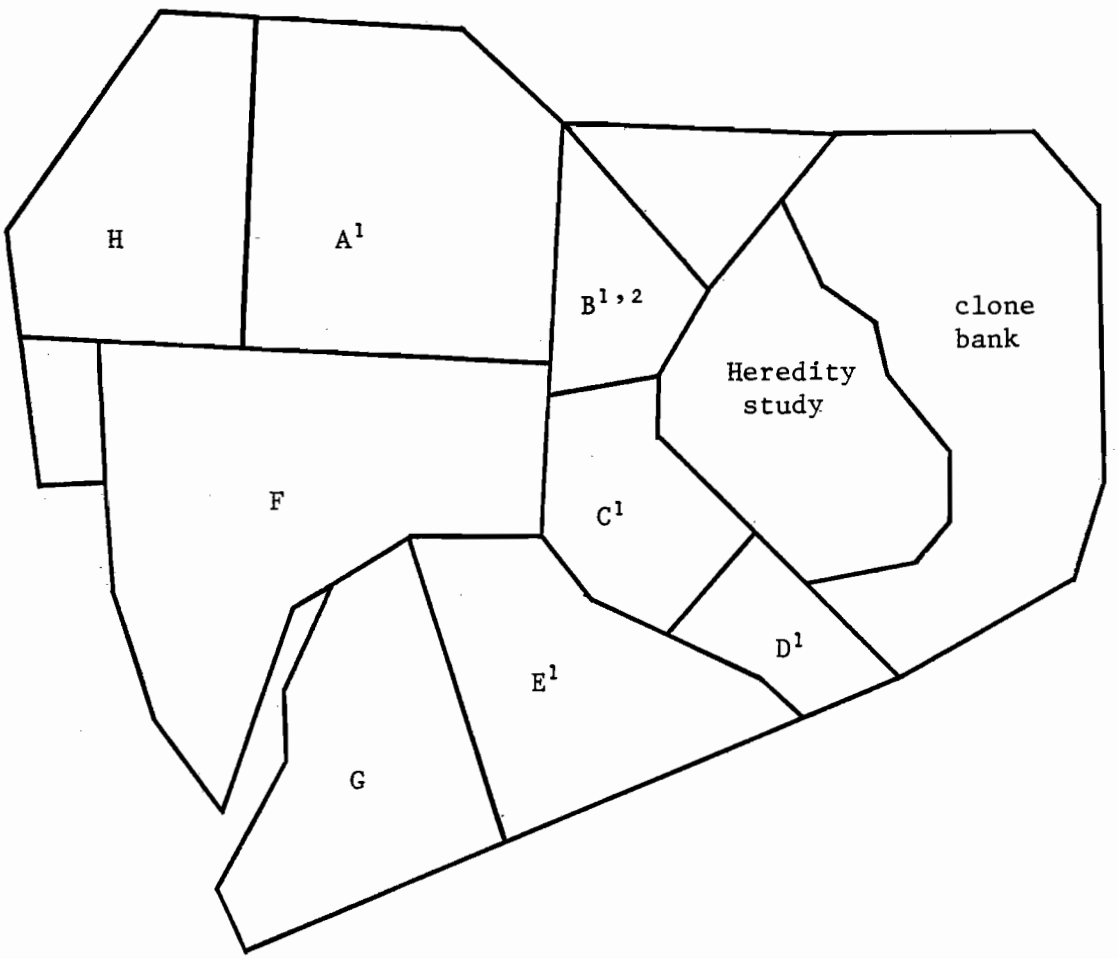

Figure 1. The location of orchard blocks (A-H) at the D. T. Mason Seed Orchard.

[1]Denotes blocks in which ramets were sampled in 1980.

[2]Denotes block in which cones were counted in 1982.

Table 1. Initial years of grafting[1], composition of clones and ramets in 1980, and size of each block in the D.T. Mason Seed Orchard.

Orchard block	Initial grafting (years)	Size (hectares)	Composition	
			Clones[2]	Mean number of ramets per clone (range)
A	1960	2.8	18 LOW, 7 MID	5.8(1 - 15)
B	1965-1966	1.1	21 LOW, 3 MID	5.6(1 - 12)
C	1961-1962	1.2	21 LOW, 5 MID	6.2(1 - 14)
D	1965-1966	1.2	21 LOW, 5 MID	5.0(1 - 14)
E	1964-1966	2.0	9 LOW, 18 MID, 7 HIGH	10.2(1 - 36)
F	1960-1966[3]	2.1	20 LOW, 7 MID, 5 HIGH	13.4(1 - 41)
G	1965-1966	1.8	4 MID, 15 HIGH	17.7(4 - 38)
H	1965-1966	3.8	4 MID, 15 HIGH	33.4(8 - 78)

[1]Years when original clones were grafted into the orchard; does not include grafting in later years to replace ramets that died.

[2]All clones are from ortets in natural stands on the west side of the central Cascade mountains of Oregon. LOW, MID, and HIGH means that ortets were from low (< 600 M), mid (600 to 900M) and high (> 900M) elevations, respectively.

[3]While grafting was initiated in block F in 1960, the block was not fully grafted until 1966, so that on the average, the ramets of block A were the oldest in the orchard.

Table 2. Population samples for which single and multiple locus estimates of the proportion of outcrossed progeny (t) were obtained.

Progeny Population	Designation
Six clones:	
A. Pruned ramets (PR) - Upper crown (U)	PR-U
B. Pruned ramets - Lower crown (L)	PR-L
C. Pruned ramets - U and L combined	PR
D. Nonpruned ramets (NP) - Upper crown	NP-U
E. Nonpruned ramets - Lower crown	NP-L
F. Nonpruned ramets - U and L combined	NP
G. Upper crown - PR and NP combined	U
H. Lower crown - PR and NP combined	L
I. Combined over P, NP, U and L	Overall
24 clones:	
J. Total block A	24 clones (block A)

Table 3. Estimates[1] of detection probabilities (G_i), outcrossing rates (t_{m_i}), and variances of t_{m_i} ($s^2_{\hat{t}_{m_i}}$) for the upper (U) crown position of six pruned (PR) seed orchard clones, based on pollen pool allelic frequencies derived from independent and combined (in brackets) data sets.

Clone	N	$\hat{G}_i$	$\hat{t}_{m_i}$	$s^2_{\hat{t}_{m_i}}$
PR-160-U	66	0.526 [0.522]	0.778 [0.784]	0.019 [0.019]
PR-163-U	65	0.708 [0.705]	0.847 [0.851]	0.009 [0.009]
PR-168-U	85	0.567 [0.576]	0.810 [0.797]	0.013 [0.013]
PR-169-U	66	0.846 [0.855]	0.967 [0.957]	0.004 [0.003]
PR-173-U	73	0.520 [0.539]	1.186 [1.144]	0.016 [0.014]
PR-199-U	67	0.791 [0.772]	0.717 [0.734]	0.006 [0.007]

[1]Estimates based on progeny data collected at five loci: LAP1, GOT3, GLYDH, CAT, and DIA.

Table 4. Form for the analysis of variance of filled seed proportions.

Source of variation	d.f	Expected mean squares[1]
Pruning (A)	a-1	$\sigma^2 + d\sigma^2_{C(AB)} + cd\sigma^2_{AB} + bcd\sigma^2_{\delta} + bcdK^2_A$
Clones (B)	b-1	$\sigma^2 + d\sigma^2_{C(AB)} + acd\sigma^2_B$
A x B	(a-1)(b-1)	$\sigma^2 + d\sigma^2_{C(AB)} + cd\sigma^2_{AB}$
Ramets (C) in A x B	(c-1)(ab)	$\sigma^2 + d\sigma^2_{C(AB)}$
Crown position (D)	d-1	$\sigma^2 + ac\sigma^2_{BD} + abcK^2_D$
A x D	(a-1)(d-1)	$\sigma^2 + c\sigma^2_{ABD} + bcK^2_{AD}$
B x D	(b-1)(d-1)	$\sigma^2 + ac\sigma^2_{BD}$
A x B x D	(a-1)(b-1)(d-1)	$\sigma^2 + c\sigma^2_{ABD}$
[C in A x B] x D	[(c-1)(ab)](d-1)	σ^2

[1] σ^2 = variance due to interactions between crown positions and ramets within clones and pruning treatments.

σ^2_{ABD} = variance due to interaction of pruning treatments, clones, and crown positions.

σ^2_{BD} = variance due to interaction of clones and crown positions.

K^2_{AD} = fixed effects interaction of pruning treatments and crown positions.

K^2_D = fixed effect of ith crown position.

$\sigma^2_{C(AB)}$ = variance due to differences among ramets within pruning treatments and clones.

σ^2_{AB} = variance due to interaction of pruning treatments and clones.

σ^2_B = variance due to differences among clones.

σ^2_{δ} = variance due to differences among pruned and nonpruned ramets which are not accounted for by pruning treatments.

K^2_A = fixed effect of the ith pruning treatment.

Table 5. Form for the analysis of variance of transformed ($logs_{10}$) cone numbers.

Source	d.f.	Expected mean square [1]
Clones (B)	b-1	$\sigma^2 + d\sigma^2_B$
Crown levels (D)	d-1	$\sigma^2 + bK^2_D$
Error	(b-1)(d-1)	σ^2

[1] σ^2 = variance due to differences between clones and crown positions.

K^2_D = fixed effect of the ith crown position.

σ^2_B = variance due to differences among clones.

Table 6. Single locus estimates (for 5 loci) of average proportions of random outcrossing (t) in nine progeny populations of six clones, and from one progeny population of 24 clones, in block A of the D.T. Mason Seed Orchard (standard errors in parentheses).

Progeny population[1]	N	Locus					Unweighted mean	Heterogeneity chi-square[4] (df)
		LAP1[2]	GOT3[3]	GLYDH[3]	CAT[3]	DIA[3]		
A. PR-U	422	0.953(.064)	1.018(.083)	0.964(.095)	0.651(.122)	1.052(.089)	0.928	8.01(4)
B. PR-L	364	1.006(.069)	1.175(.067)	0.696(.101)	0.719(.128)	1.171(.091)	0.953	23.86(4)**
C. PR (U and L)	786	0.975(.047)	1.087(.056)	0.840(.070)	0.682(.088)	1.107(.064)	0.938	23.02(4)**
D. NP-U	415	0.954(.066)	0.912(.098)	1.092(.097)	1.032(.120)	1.036(.085)	1.005	2.43(4)
E. NP-L	263	0.787(.086)	0.825(.125)	1.181(.125)	0.747(.131)	1.232(.107)	0.955	17.26(4)**
F. NP (U and L)	678	0.892(.052)	0.877(.078)	1.109(.076)	0.927(.089)	1.112(.067)	0.983	11.65(4)*
G. U (PR and NP)	837	0.953(.046)	0.964(.065)	1.029(.068)	0.839(.086)	1.043(.061)	0.966	4.60(4)
H. L (PR and NP)	627	0.914(.054)	1.005(.072)	0.889(.079)	0.738(.093)	1.198(.070)	0.948	19.31(4)**
I. Overall	1464	0.938(.035)	0.981(.048)	0.967(.052)	0.797(.063)	1.109(.046)	0.958	17.61(4)**
J. 24 clones (total block A)	419[6]	0.954(.050)	1.025(.047)[2]	0.764(.079)	0.932(.062)	0.983(.066)	0.960[5]	21.25(9)*

[1]Designations are described in Table 2.
[2]Single locus estimate based on 3 alleles.
[3]Single locus estimate based on 2 alleles.
[4]Heterogeneity of t estimates over loci.
[5]Mean estimate of outcrossing for block A clones based on 10 loci. Additional loci and $\hat{t}$ values at these loci were: PGM1 (1.046), LAP2 (0.994), GOT2 (1.030), 6PGD (0.931) and IDH (0.943). The heterogeneity chi-square value was computed over the 10 loci.
[6]Among the 10 loci scored for population J, the mean number of observations per locus was 419 (range 403 to 426).

*Significant at 0.05 probability level
**Significant at 0.01 probability level

Table 7. Estimated allelic frequencies at five allozyme loci in the outcross pollen pools of five progeny populations (PR-U, PR-L, NP-U, NP-L, overall) of six clones, and in one progeny population of 24 clones, in block A of the D. T. Mason Seed Orchard.

Locus	Allele	Progeny population				Heterogeneity chi-square[1] (df)	Progeny poulation		Heterogeneity chi-square[2] (df)
		PR-U	PR-L	NP-U	NP-L		Overall (six clones)	Block A (24 clones)[3]	
LAP1	2	0.52	0.53	0.51	0.52		.52	0.57	
	5	0.19	0.20	0.23	0.21		.21	0.18	
	7	0.30	0.28	0.25	0.27		.27	0.25	
	n	422	364	415	263	2.15(6)	1464	425	3.70(2)
GOT3	1	0.11	0.11	0.14	0.15		0.13	0.11	
	2	0.89	0.89	0.86	0.85		0.87	0.89	
	n	422	364	415	263	3.11(3)	1464	422	1.43(1)
GLYDH	2	0.60	0.54	0.60	0.55		0.58	0.58	
	3	0.40	0.46	0.40	0.45		0.42	0.42	
	n	422	364	415	263	2.77(3)	1464	416	0.00(1)
CAT	1	0.32	0.35	0.31	0.21		0.30	0.21	
	2	0.68	0.65	0.69	0.79		0.70	0.79	
	n	422	364	415	263	5.68(3)	1464	421	9.00(1)**
DIA	1	0.23	0.20	0.22	0.22		0.22	0.22	
	3	0.77	0.80	0.78	0.78		0.78	0.78	
	n	422	364	415	263	0.95(3)	1464	403	0.00(1)

[1]Heterogeneity of allelic frequency estimates over PR-U, PR-L, NP-U, and NP-L.
[2]Heterogeneity of allelic frequency estimates between overall (six clones) and 24 clones.
[3]Allelic frequencies for the five remaining loci are [locus (allele-frequency)]: PGM1 (1-0.07, 2-0.91, 3-0.02) LAP2 (3-0.96, 5-0.04) GOT2 (1-0.06, 2-0.93, 3-0.02) 6PGD (1-0.02, 2-0.96, 3-0.03) IDH (1-0.12, 2-0.80, 3-0.08).

**Significant at 0.01 probability level.

Table 8. Single clone (t_{m_i}) and population (t_m, data for all clones combined) multilocus estimates of proportions of outcrossed progeny for the various combinations of upper (U) and lower (L) crown positions of pruned (PR) and nonpruned (NP) ramets of six Douglas-fir seed orchard clones, and population estimate of t_m for 24 clones in block A (standard errors in parentheses).

	Single Clone Estimates															
	Clone															
	160		163		168		169		173		199					
Progeny samples[1]	$\hat{t}_{m_i}$	n	$\hat{t}_{m_i}$	n	$\hat{t}_{m_i}$	n	$\hat{t}_{m_i}$	n	$\hat{t}_{m_i}$	n	$\hat{t}_{m_i}$	n	Heterogeneity chi-square[2]	Unweighted mean	Population estimates
PR-U	0.862(.094)	66	0.923(.069)	65	0.974(.077)	85	0.949(.048)	66	1.132(.071)	73	0.817(.067)	67	11.85*	0.943(0.030)	0.933(0.026)
PR-L	0.565(.101)	50	0.457(.077)	57	0.952(.083)	71	0.955(.046)	65	1.105(.076)	69	0.856(.071)	52	51.48**	0.815(0.032)	0.832(0.030)
PR (U and L)	0.732(.070)	116	0.703(.055)	122	0.963(.057)	156	0.953(.033)	131	1.118(.053)	142	0.835(.049)	119	40.52**	0.884(0.022)	0.888(0.020)
NP-U	0.812(.094)	69	1.080(.049)	67	0.855(.089)	63	1.053(.028)	72	0.924(.081)	70	0.920(.055)	74	15.63**	0.940(0.029)	0.977(0.024)
NP-L	0.484(.108)	48	--	12	0.824(.105)	54	1.079(.027)	47	0.863(.095)	56	0.886(.075)	46	38.46**	0.827[3](0.039)	0.901(0.035)
NP (U and L)	0.679(.073)	117	1.070(.046)	79	0.837(.068)	117	1.062(.020)	119	0.897(.062)	126	0.905(.045)	120	43.98**	0.908(0.023)	0.949(0.020)
U (PR and NP)	0.837(.067)	135	1.004(.043)	132	0.926(.058)	148	1.003(.028)	138	1.029(.055)	143	0.872(.043)	141	12.83*	0.945(0.021)	0.953(0.018)
L (PR and NP)	0.525(.074)	98	0.559(.073)	69	0.900(.066)	125	1.009(.030)	112	0.998(.062)	125	0.872(.052)	98	63.94**	0.810(0.025)	0.861(0.023)
Overall	0.707(.051)	233	0.849(.041)	201	0.914(.045)	273	1.006(.020)	250	1.014(.042)	268	0.872(.033)	239	43.71**	0.894(0.016)	0.915(0.014)
24 clones[4]	--		--		--		--		--		--		--	--	0.935(0.026)

[1]Designations are described in detail in Table 2.
[2]Heterogeneity of t_{m_i} estimates over clones [with 5 degrees of freedom (df) for all test except that for NP-L (4df)].
[3]Clone 163 not included in mean.
[4]Population estimate of t_m based on progeny data from 24 of the 25 clones in Mason block A (ramets were all pruned).

*Significant at 0.05 probability level.
**Significant at 0.01 probability level.

Table 9. Heterogeneity chi-square analysis of mean (unweighted) differences in multilocus t_{m_i} values between upper and lower crown positions and pruned (PR) and nonpruned (NP) ramets of six Douglas-fir clones.

Treatment comparison	Heterogeneity χ^2 (1 df)
Upper vs. lower crowns:	
PR-U vs. PR-L	8.76**
NP-U vs. NP-L	5.49*
U vs. L (PR and NP combined)	17.26**
Pruned vs. nonpruned ramets:	
NP-U vs. PR-U	0.00
NP-L vs. PR-L	0.06
PR vs. NP (U and L combined)	0.60

*Significant at 0.05 probability level.
**Significant at 0.01 probability level.

Table 10. Analysis of variance of filled seed proportions.

Source	d.f.	Mean square	F-ratio[1]
Pruning level (A)	1	0.01537	0.55
Clone (B)	5	0.32512	12.86**
AxB	5	0.02810	1.11
Ramet (C) in A x B	21[2]	0.02529	
Crown position (D)	1	0.31205	8.68*
A x D	1	0.00333	0.07
B x D	5	0.03597	1.47
A x B x D	5	0.04924	2.02
D x [C in A x B]	21[2]	0.02444	
Total	65		

[1]All F tests are approximate due to the effect of missing value estimation on the expected mean squares.

[2]Due to missing data, degrees of freedom for each error mean square are reduced by 3 (from the original 24 degrees of freedom).

*Significant at .05 probability level.
**Significant at .01 probability level.

Table 11. Proportions[1] of filled seeds in cones of upper (U) and lower (L) crowns of pruned (PR) and nonpruned (NP) ramets of six Douglas-fir seed orchard clones.

Pruning treatment	Crown position	Clone 163	173	160	168	199	169	Mean
PR	U	0.323	0.422	0.446	0.689	0.724	0.420	0.504
PR	L	0.218	0.545	0.245	0.597	0.337	0.211	0.359
PR (U and L)		0.271	0.484	0.346	0.643	0.531	0.316	0.432
NP	U	0.565	0.688	0.223	0.696	0.675	0.271	0.520
NP	L	0.130	0.600	0.288	0.590	0.567	0.235	0.402
NP (U and L)		0.348	0.644	0.256	0.643	0.621	0.253	0.461
U (PR and NP)		0.444	0.555	0.335	0.692	0.700	0.346	0.512
L (PR and NP)		0.174	0.573	0.267	0.593	0.452	0.223	0.381
Overall mean		0.309	0.564	0.301	0.643	0.576	0.285	0.446

[1]Unweighted mean proportions of filled seeds (based on X-ray analysis) from three ramets (number of seeds per ramet x pruning treatment x crown position combination ranged from 42 to 275 (mean 198)).

Table 12. Analysis of heterogeneity of filled seed proportions (PF) over six Douglas-fir seed orchard clones, for cones collected from the upper (U) and lower (L) crown positions of pruned (PR) and nonpruned (NP) ramets.

Pruning treatment-crown position	N	χ^2 (5 df)
PR-U	3407	252.27**
PR-L	3598	381.76**
PR (U and L)	7005	457.09**
NP-U	3087	536.37**
NP-L	2958	382.99**
NP (U and L)	6045	845.57**
U (PR and NP)	6494	602.79**
L (PR and NP)	6556	724.20**
OVERALL	13050	1213.14**

**Significant at .01 probability level.

Table 13. Heterogeneity chi-square analysis of filled seed proportions between cones from upper (U) and lower (L) crown positions and from pruned (PR) and nonpruned (NP) ramets of six clones in the D.T. Mason Seed Orchard.

Treatment comparison	Clone[1] 163 χ^2	n	173 χ^2	n	160 χ^2	n	168 χ^2	n	199 χ^2	n	169 χ^2	n	All clones combined[1] χ^2	n
Upper vs. lower crown:														
PR-U vs. PR-L	39.19**	998	18.52**	1203	53.74**	1204	11.07**	1201	180.72**	1202	60.48**	1197	193.45**	7005
NP-U vs. NP-L	83.40**	400	10.13**	1200	4.45*	800	14.70**	1201	8.22**	1087	0.26	1357	38.97**	6045
U vs. L (PR and NP)	109.37**	1398	0.79	2403	21.18**	2004	25.64**	2402	145.48**	2289	34.26**	2554	211.91**	13050
Pruned vs. Nonpruned ramets:														
NP-U vs. PR-U	14.25**	598	86.81**	1203	52.10**	1002	0.07	1202	3.44	1202	34.29**	1286	0.01	6494
NP-L vs. PR-L	7.37**	800	3.71	1200	2.30	1001	0.06	1200	70.04**	1087	1.84	1268	46.76**	6556
PR vs. NP (U and L)	4.20*	1398	63.33**	2403	18.65**	2004	0.00	2402	26.86**	2289	14.16**	2554	25.31**	13050

[1]All chi-square values have one degree of freedom.

*Significant at .05 probability level.
**Significant at .01 probability level.

Table 14. Observed proportions of cones at three crown levels in six Douglas-fir seed orchard clones.[1]

	Crown level			
Clone	Upper	Middle	Lower	N
167	0.71	0.21	0.08	197
172	0.77	0.20	0.03	491
173	0.34	0.62	0.04	733
208	0.29	0.34	0.37	231
209	0.38	0.56	0.07	93
347	0.27	0.43	0.30	67
Overall clones	0.49	0.42	0.09	
N	887	754	171	1812

[1]Each clone represented by a single ramet.

Table 15. Analysis of variance of cone number ($\log_{10}$) in three crown levels of six Douglas-fir seed orchard clones.

	d.f.	Mean square	F-ratio
Clone	5	0.3372	2.66
Crown position	2	0.7900	6.22*
Error	10	0.1270	

*Significant at 0.05 probability level.

Table 16. Number of flowering ramets (NFR) and estimated flowering productivity (FLP)[1] for six Douglas-fir seed orchard clones in block A.

Clone	NFR	FLP[1]	
		Male	Female
160	10	500,000	3,065
163	27	1,350,000	8,100
168	4	200,000	2,000
169	28	1,355,000	14,000
173	26	1,300,000	13,000
199	16	705,500	7,550

[1]Total number of flowers.

Table 17. Estimated productivity values of Douglas-fir plantations at age 10, established with wind-pollinated progenies from the upper, lower, and total crowns of six clones and from 24 clones in block A.

Treatment class	Productivity value
Six clones:	
Upper crown	0.96
Lower crown	0.87
Total crown	0.93
24 clones:	0.95

Table 18. Mean estimates of proportions of filled seed (PF), proportion of viable selfed progeny (s), productivity values (PV), and percent contributions to the total cone harvest, for the upper, mid[1], lower, and total[2] crown positions of six Douglas-fir seed orchard clones.

Crown position	$\widehat{PF}$	$\hat{s}$	$\widehat{PV}$	% cone contribution
Upper	0.51	0.06	0.96	49
Mid	0.45	0.12	0.91	42
Lower	0.38	0.19	0.87	9
Total crown	0.47	0.10	0.93	100

[1]All mid crown estimates, except % cone contribution, were derived by averaging upper and lower crown estimates.

[2]Total crown estimates of PF, s, and PV represent averages weighted by the proportional cone contributions from the upper, mid, and lower crown positions.

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