GENETICS OF DOUGLAS-FIR SEED ORCHARDS: EXPECTATIONS AND REALITIES

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Abstract.--This paper summarizes the results of a decade of research conducted on Douglas-fir seed orchards in British Columbia, Canada. Firstly, the seed orchard's basic assumptions (i.e., reproductive synchrony, parental balance, minimal inbreeding, isolation, and random mating) were systematically examined in clonal and seedling orchards. Secondly, the impact of two crop management techniques, supplemental-mass-pollination and overhead cooling, on the genetic efficiency (quality and quantity) of orchards were assessed in a controlled designed experiment. Thirdly, several management practices that are commonly used in British Columbia were evaluated. These include the reliance on cone count as a sole criterion for determining parental balance, the management of crop trees only, the removal of non- or low-seed cone producing trees, and the normally accepted loss of some seeds from a few trees as a prerequisite to cone harvesting. Finally, a novel approach to genetic thinning that optimizes both genetic gain and diversity as well as maintaining flexibility is proposed.

INTRODUCTION

Seed orchards represent the link between breeding programs and reforestation activities through the consistent delivery of abundant crops of genetically improved seed to meet a predetermined need. The attainment of genetic gain is dependent upon maintaining the same frequency of desirable genes in the orchard seed crops at the same level that is present in the selected population. The maintenance of similar allele frequencies between the seed orchard population and its seed crops requires attainment of the random-mating assumption of the Hardy-Weinberg theorem.

Random mating in seed orchards (i.e., panmictic equilibrium) can only be achieved if the genetic entities (i.e., clones or

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families) are in reproductive synchrony and have similar reproductive output (i.e., parental balance). In addition, since coniferous forest tree species are mainly wind-pollinated and often display strong inbreeding depression, the potential of pollen migration (i.e., contamination) from extraneous undesirable sources and inbreeding through self-fertilization and/or consanguineous mating is of concern. Therefore, a seed orchards genetic efficiency, defined as the degree to which the orchard crop reflects the genetic superiority and diversity present in the orchard population, is dependent upon maximizing reproductive synchrony and output equality and minimizing inbreeding and contamination. In short, a seed orchard must represent a nearly perfect, closed, panmictic population.

Due to their importance, seed orchards and their genetics are the focus of increased research activities. Their robustness to violation of the above-mentioned conditions has been assessed for several species. It is not surprising that most of these conditions are not fully met and that several management options were proposed or implemented. Supplemental-mass-pollination (SMP), the broadcast application of viable pollen to non-isolated receptive strobili (Wakeley _et al._ 1966), is the most common crop-management method used by seed orchard managers. Overhead cooling of orchard trees, another crop-management practice, was proposed as a means of reducing orchard contamination by delaying reproductive bud development relative to background pollen sources (Silen and Keane 1969). Although its use is restricted to the Pacific Northwest, the cooling treatment is gaining increased attention as a viable method for reducing pollen contamination.

This paper summarizes the results of research conducted in Douglas-fir [_Pseudotsuga menziesii_ (Mirb.) Franco] seed orchards which systematically examined basic seed orchard assumptions and their inter-relationships. In addition, the impact of SMP and overhead cooling methods on the genetic efficiency of seed orchards is evaluated.

REVIEW OF DOUGLAS-FIR SEED ORCHARD GENETIC RESEARCH

Reproductive Phenology

Synchronization of the phenology of reproductive strobili among different clones or families is a fundamental need for any successful seed orchard operation. Failure of synchronization affects other, if not all, assumptions. It is well known that both time and temperature (summarized as heat sums) have a significant effects on flowering time (Kramer and Kozlowski 1979). Since seed orchards are established with clones or selections that may require different heat sums, it is to be expected that time differences of reproductive phenology will be found.

Reproductive bud phenology information for every sexually active tree in a high-elevation Douglas-fir seed orchard was collected for six years. Each tree was monitored on alternate days throughout the pollination season. The critical stage of seed-cone receptivity and pollen shedding was recorded following the classification proposed by Ho (1980) and Owens et al. (1981). In addition, background pollen was monitored using pollen traps (for more details see Fashler and Devitt 1980). The six year study encompassed three years when the flowers opened under natural conditions and another three years under overhead cooling. Differences were found in both the length of pollination period and the number of genotypes reproductively active at a given time (Fashler and El-Kassaby 1987). Figure 1 presents a typical pattern of the reproductive phenology and timing in the seed orchard versus background pollen under both treatments. When the seed orchard was not cooled, the female receptivity and pollen release periods averaged 24.3 and 27.7 days, respectively. Under cooling, the pollination period was reduced by an average of seven days to 17.3 days for females and 18.3 for males. In addition, the maximal number of flowering females and male occurred later than the local pollen flight and developed in a relatively shorter time in the cooled years compared to years under natural conditions (Fig. 1).

Differences in the number of receptive females and pollen-shedding males were apparent at various stages (i.e., early, intermediate and late) in the pollination period in both cooled and uncooled years (Fig. 1). Cooling appears to have reduced greatly the magnitude of these differences. The disproportion in females and males suggests potential differences in the filled seed produced in each phenological class due to lack of pollen and/or availability of related pollen (El-Kassaby et al. 1984; Woods and Heaman 1989).

The length of pollination period and its differentiation into early, intermediate and late reproductive phenology classes suggest that a major deviation from panmictic equilibrium exists. Since females are receptive for only four to six days and the optimal duration of pollen shedding varies between three to five clays, mating between early- and late-flowering trees is not possible. Then the effective breeding population size is reduced and a continum of small breeding populations throughout the pollination season will be the result, as opposed to one panmictic population.

Reproductive phenological differences can affect all seed-orchard assumptions in several ways. Under panmixia, the expected selfing rate is equal n/n^2, where n is the number of clones. Therefore, in a hypothetical seed orchard that consists of 30 clones, the expected selfing rate is 3.33%. If this orchard is divided into three non-overlapping breeding sub-populations with equal proportions (1:1:1) or unequal proportions (1:4:1) of clones. The expected selfing rates for these situations will be 10 and 6.67%, respectively. This

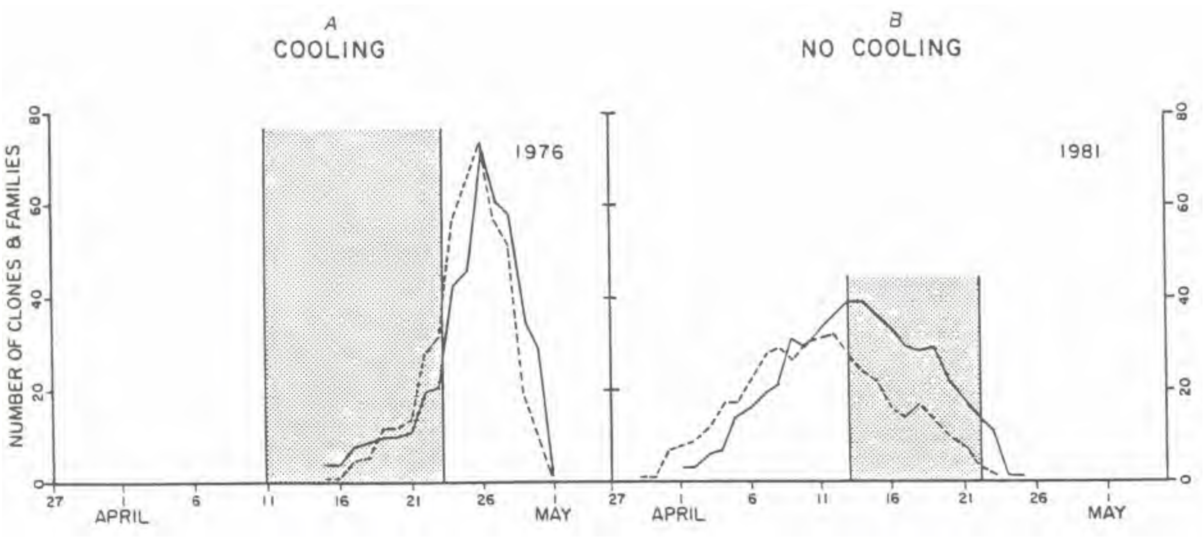

Fig. 1. Reproductive phenology comparison between (A) cooled (1976) and (B) uncooled (1981) years indicating the timing of the local background pollen flights.

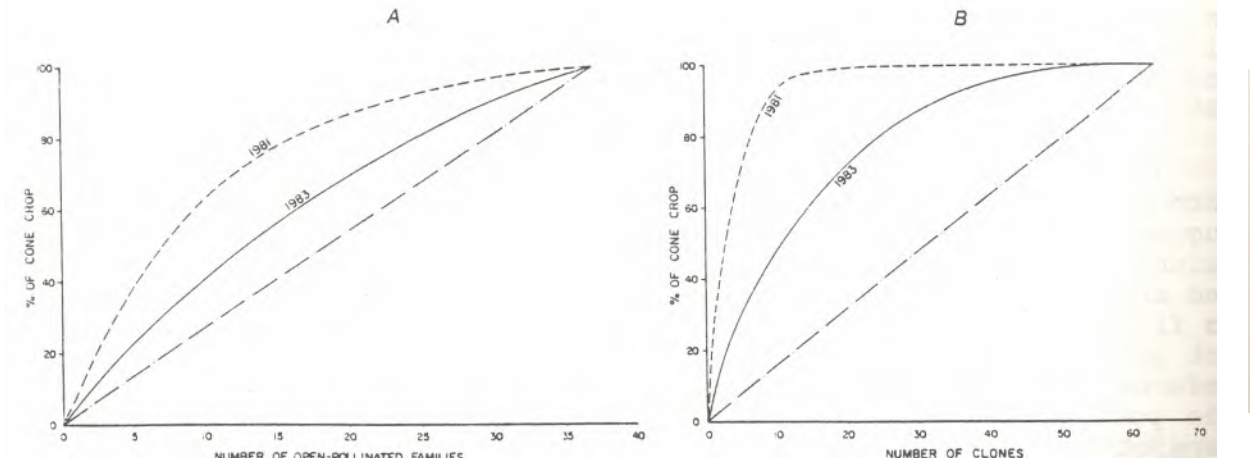

Fig. 2. Cumulative seed-cone production curves for open-pollinated families (A) and clones (B) for a good (1983) and a poor (1981) cone year. Straight line represents equal contribution.

hypothetical orchard produced three- and two-fold differences in selfing expected when panmictic disequilibrium existed.

The frequency and timing of receptive females and pollen-shedding males also affects the rate of pollen contamination. If the background pollen is high in early or late phenology classes, it is expected that most of the receptive sites will be saturated by background unrelated pollen (El-Kassaby and Ritland 1986a).

Reproductive phenological differences also affect the amount of gametic contribution by every clone to the resultant seed crop. A high seed or pollen producing clone in the early or late reproductive phenology classes could contribute the same as or less than the amount contributed by a low or medium reproductive output clone during the height of pollination (El-Kassaby and Askew 1989).

Variation in reproductive developmental phases observed in one Douglas-fir seed orchard was not associated with variation in the timing of seed maturation, therefore, cone harvesting can begin irrespective of flowering phenology, before cone opening starts (Edwards and El-Kassaby 1988). The results from the flowering phenology and date-of-cone-collection study indicated that seeds in all phenological classes had matured by the time they were harvested, and that seed-ripening mechanisms have adapted to reach completion over different lengths of time (Edwards and El-Kassaby 1988).

Reproductive Output

The maintenance of similar allelic frequencies between a seed orchard and its seed crop is dependent upon the presence of balanced gametic production among the orchard's genetic entities. Due to the ease of estimating clonal seed-cone production, seed orchards' parental balance often are based solely on seed-parent contributions. In fact, the so-called "20/80" rule (i.e., 20% of the clones produce 80% of the cone crop) was coined by the North Carolina State Tree Improvement Co-operative in 1976 (Anon. 1976). Since then, parental balance values based on seed-parent contributions have become an accepted method to evaluate and even to provide genetic rating of seed orchard crops.

i) Parental balance based on seed-cone production

In an attempt to estimate the parental balance of a clonal/seedling Douglas-fir seed orchard, individual tree cone-counts were conducted at harvest over the 1976-1983 period. Variation in cone production among families and clones over the eight-year-period revealed the following: 1) seedling trees' cone crop consistently exceeded that of clonal trees despite physiological maturity differences, 2) a biennial cone-bearing trend was apparent, notwithstanding an overall increase in production with age, 3) clones and families were classified as consistently high, consistently low or inconsistent cone producers, suggesting that both genetic and environmental factors influence cone production, and 4) parental balance was largely affected by the crop size and genetic classes (Fig. 2a,b).

Parental balance of clones and families was assessed by cumulative cone-yield curves following the method of Griffin (1982). Parental contribution varied substantially among years and between genetic classes (Fig. 2a,b). In a good cone year (1983), approximately 50% of the families produced 63% of the total cone crop, a near-ideal situation. However, in a poor cone year (1981), the top 50% of the families produced 83% of the total crop, with the remaining 50% producing only 17% of the total crop (Fig. 2a). The clonal trees' production was more distorted than that of families in both good and poor cone years (Fig. 2b). In 1981, 10% of the clones produced 80% of the crop and in 1983, 50% of the clones produced 88% of the crop total as compared to 63% for families.

Various crop-management options have been proposed to adjust for the observed imbalance in cone crops. These include SMP (El-Kassaby et al. 1986a, 1989c; El-Kassaby and Reynolds 1989; Reynolds and El-Kassaby 1989), mixing seed crops from several years (Varnell et al. 1967; Schmidtling 1983; El-Kassaby et al. 1986a, 1989c), abortion of small cone crops (El-Kassaby et al. 1986a; El-Kassaby and Ritland 1986a), individual-clone harvesting and seed extraction (Reynolds and El-Kassaby 1989), and selective use of cone-induction methods El-Kassaby et al. 1989c).

ii) Parental Balance Based on Seed Production

The preceding section on parental balance assumed that reproductive energy is equal to reproductive success (i.e., cone size and the number of filled seeds per cone is equal across the different parental groups). A comparison of parental balance among 30 Douglas-fir trees based on seed-cone crop to that of filled-seed crop (Reynolds and El-Kassaby 1989) demonstrated that the seed-crop data have provided a more-accurate assessment of the genetic diversity and the family representation in the resultant seed crop than did the cone crop (Fig. 3a). Parental balances of "57/90" and "40/90" were obtained for seed-cone and seed crops, respectively (Fig. 3a). In addition, the reproductive-rank order of the studied trees changed substantially when seed production was used (Fig. 3b). Five out of the top 10 cone-producing trees remained among the top 10 seed producers, while the other five dropped to "intermediate" seed production (rank 11-20). The change in rank among the 10 intermediate cone producers indicated the presence of high, intermediate and low seed producers. On the other hand, nine out of the 10 "low" cone producers maintained their relative rank as seed producers. Comparing the results of parental

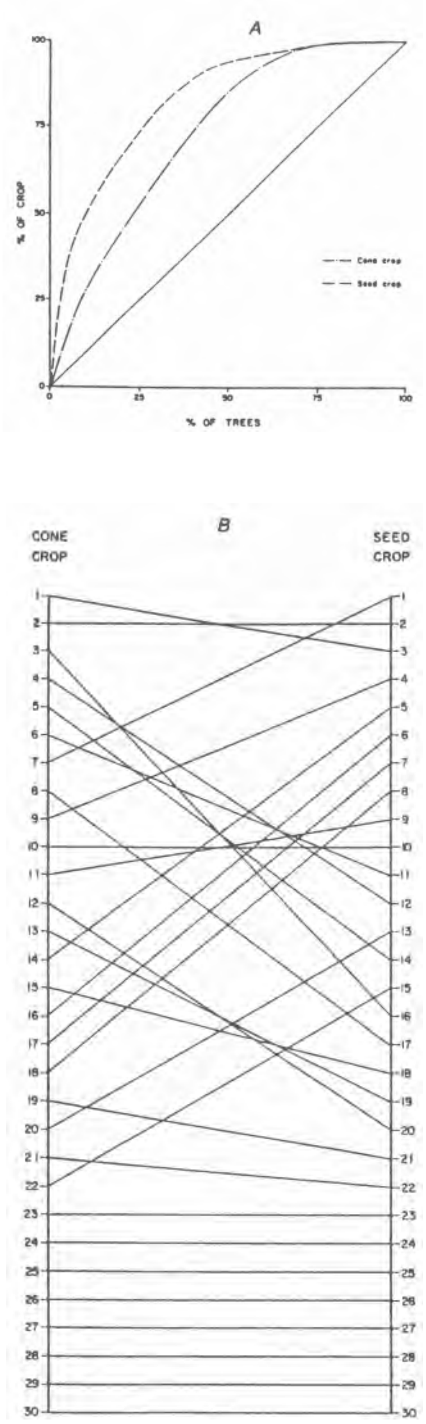

Fig. 3. (A) Cumulative cone and seed production curves for 33 Douglas-fir trees. (B) Rank order presentation for the same 30 Douglas-fir trees, based on cone and seed crops.

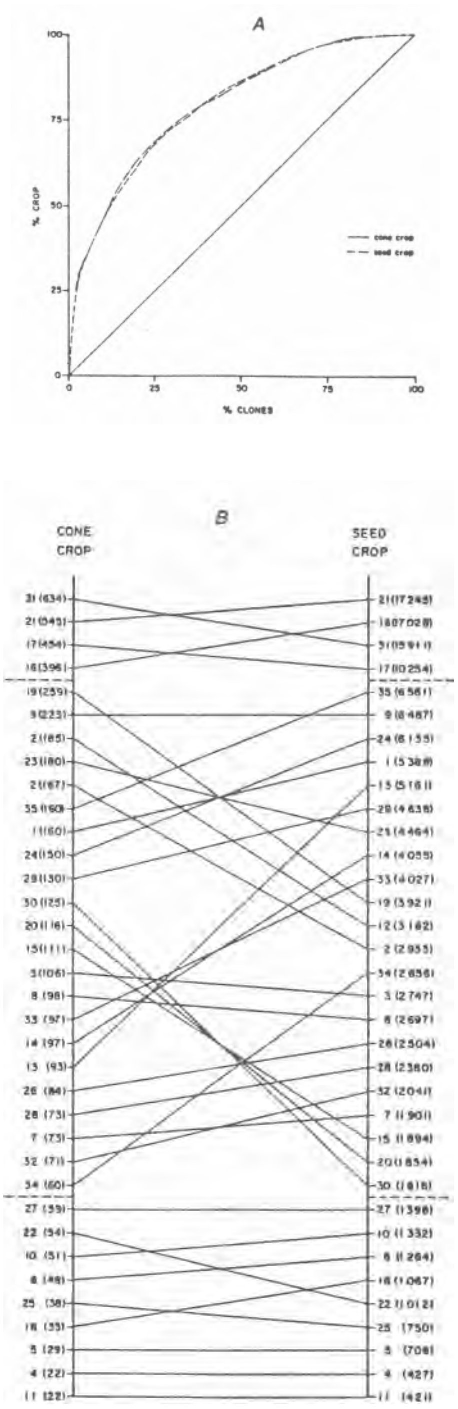

Fig. 4 (A) Cumulative cone and seed production curves for a 1.5-generation Douglas-fir seed orchard. (B) Rank order presentation of the orchard clones based on cone and seed crops.

balance based on the two approaches indicates the following: 1) the estimate based on cone harvest did not reflect the distortion in the seed crop, 2) 90% of the cone crop was obtained from 17 trees (57%), while 90% of the seed crop was obtained from 12 trees (40%) (smaller genetic base), 3) the tree that ranked number 8 in seed production was among those producing the 90% of the seed crop, but it was not among those producing 90% of the cone crop (different genetic representation) and 4) there was little rank change in the "low" cone and seed producers.

Table 1. Variation in the number of cones (C) and filled seeds (S) per ramet for the studied 35 Douglas-fir clones combined and for the three production classes (high, intermediate and low) (values are percentage of the total sum of squares).

Source of Variation	Combined			High			Intermediate			Low		
	d.f.	C	S	d.f.	C	S	d.f.	C	S	d.f.	C	S
Among Clones	34	48.2**	49.3**	3	4.6^{ns}	6.0^{ns}	21	11.0^{ns}	14.5^{ns}	8	3.3ns	58ns
Within Clones	166	51.8	50.7	21	95.4	94.0	98	89.0	85.5	47	96.7	94.2

** P<0.01; ^{ns}, not significant at P<0.05

The results obtained from this study clearly demonstrate the difference between cone versus seed crop use in Estimating parental balance. However, it failed to demonstrate the within-family or clone variation in order to determine how much of the predicted variability in performance could disappear with averaging when an entire orchard data set is considered. To address this question an additional study was conducted on a 1.5-generation Douglas-fir orchard and the within- and among-clonal variation in cone and seed crops were evaluated (El-Kassaby and Reynolds, unpublished). A similar trend was observed and changes in clonal cone and seed rank were obtained (Fig. 4a,b), but the parental-balance curves appeared to be identical (Fig. 4a). It must be emphasized that the clonal representation at any point on the two identical cone and seed yield curves is different (see Fig. 4b). Although the rate of distortion was the same in both cases, the genetic representation was different. The within-clone variation accounted for approximately 50% of the total sum of squares for both cone and seed analyses (Table 1). However, when the clones were grouped into three classes of production level (high, intermediate and low), the within-clone variation accounted for 85-97% of the total variation (Fig. 4b and Table 1). The separation of clones into three reproductive classes was based on the non-overlapping nature of clonal rank in both cone and seed production (Fig. 4b). In summary, this study demonstrated the following: 1) the presence of high within-clone variation in cone and seed production, 2) parental balance, seemingly icentical for cone and seed curves, represent different genetic contributions for cones and seed at any given point and 3) the clones' reproductive energy differed from their reproductive success.

iii) Cost of Reproduction

It has become common practice to select orchard trees with consistently high cone production for inclusion or retention during operational thinning. This makes sense from the point of view of the seed orchard manager since it should facilitate future seed production (Danbury 1971). There is a growing body of theoretical literature, however, that casts doubt on the validity of this procedure. It is known by various names, but most commonly is called the theory of life history evolution. The theory maintains that since there are finite resources available to an organism, selection that involves increasing the resource allocation to one trait will of necessity decrease resource availability elsewhere. More specifically, selection for higher reproductive output should concomitantly select for slower growth. Stearns (1977,1980) has presented mounting empirical and experimental evidence that demonstrate such trade-offs when selection is imposed on a population. A study in which 8-year cone production and growth increments (1976-1983), based on seed-cone count and annual ring width, was conducted to address this question (Barclay and El-Kassaby 1988; El-Kassaby and Barclay 1989). The population studied consisted of 29 open-pollinated (half-sib) Douglas-fir families that are growing in a seed orchard. The orchard environment is highly controlled and environmental influences were minimized, making the underlying genetic patterns more readily detectable. The presence of family structure permitted the estimation of phenotypic, genetic and environmental correlations using the analysis of variance and covariance methods specific to half-sib families (Becker 1984).

Table 2. Genetic correlations (r_g) between seed cone count and annual ring increment for 29 Douglas-fir open-pollinated Families for 1976-83.

Year	1976	1977	1978	1979	1980	1981	1982	1983
r	-0.776	-0.412	-0.586	0.181	-0.199	-0.316	-0.265	0.213
Cone Yield	324,105	21,473	7,186	50,077	75,588	51,699	8,541	33,787

Six out of the eight genetic correlations were negative (Table 2) reflecting different genetic propensities among families to either allocate their resources primarily to cone production or to growth. These negative correlations imply the existence of genetic variability in the proportional allocation of photosynthate to reproduction and growth, and that selection for cone production will result in negative selection for growth. In conclusion, this study demonstrated the presence of a tradeoff between reproduction and growth. Thus, the practice of selecting seed-orchard clones for high cone production or managing "cone crop" trees only in seed orchards may be expected to yield slower-growing progeny.

Relation Between Reproductive Phenology and Reproductive Output

An orchard's parental balance is often based solely on seed-parent contribution, with either an implicit or explicit assumption that the pollen contributions are balanced or that they are distributed in similar proportions to the seed-parent contributions (i.e., high, positive and significant correlation between male and female reproductive energy and success is present). Such an assumption places too little emphasis on 50% of the gametic pool (i.e., male pool) and the differential viability and selection among gametic sources. It should be emphasized that the gametic contribution by high or low pollen-producing clones or families is influenced by their reproductive timing in relation to other clones or families in the seed orchard. High pollen-producing clones during the early or late reproductive period could have similar gametic contributions to "low" clones during the height of pollination. Therefore, evaluation of the reproductive phenology and output level, for both male and female strobili, concurrently over the entire orchard population will provide a more realistic assessment of individual clones' contribution to the gametic pool.

The relationship between reproductive phenology and output in determining the gametic contribution by family was studied for 97 full-sib families in a 15-year-old Douglas-fir seed orchard (El-Kassaby and Askew 1989). Individual family gametic contribution was determined after considering: 1) the time of seed-cone receptivity, 2) time of pollen shed, 3) the level of seed-cone production, 4) the level of pollen production and 5) the reproductive phenology overlap between each family and all other families in the orchard. The study demonstrated that any evaluation based on reproductive phenology (Fig. 5a) and parental balance curves (Fig. 5b) alone is limited and that their concurrent use is more informative in obtaining an accurate assessment of each family's potential gametic contribution to the orchard crop. The study indicated that some of the top seed or pollen producers' gametic contributions were surpassed by lower ones when reproductive phenology was considered. The method presented could be used also as a management tool to direct SMP and/or cone-induction activities towards the low gametic contributors to adjust parental gametic balance toward equality.

Pollen Contamination

The maintenance of similar allelic and genotypic frequencies between a seed-orchard population and its seed crop is dependent

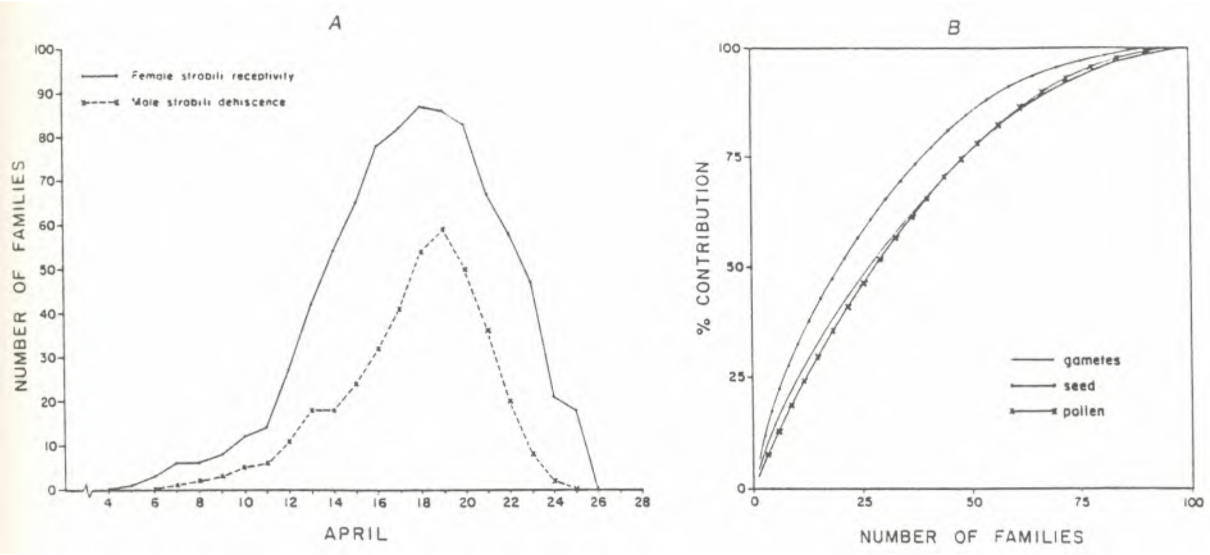

Fig. 5 (A) Reproductive phenology for a 15-year-old Douglas-fir seedling seed orchard. (B) Estimates of cumulative seed and pollen cone and gamete contributions for seed crop from 5 (A).

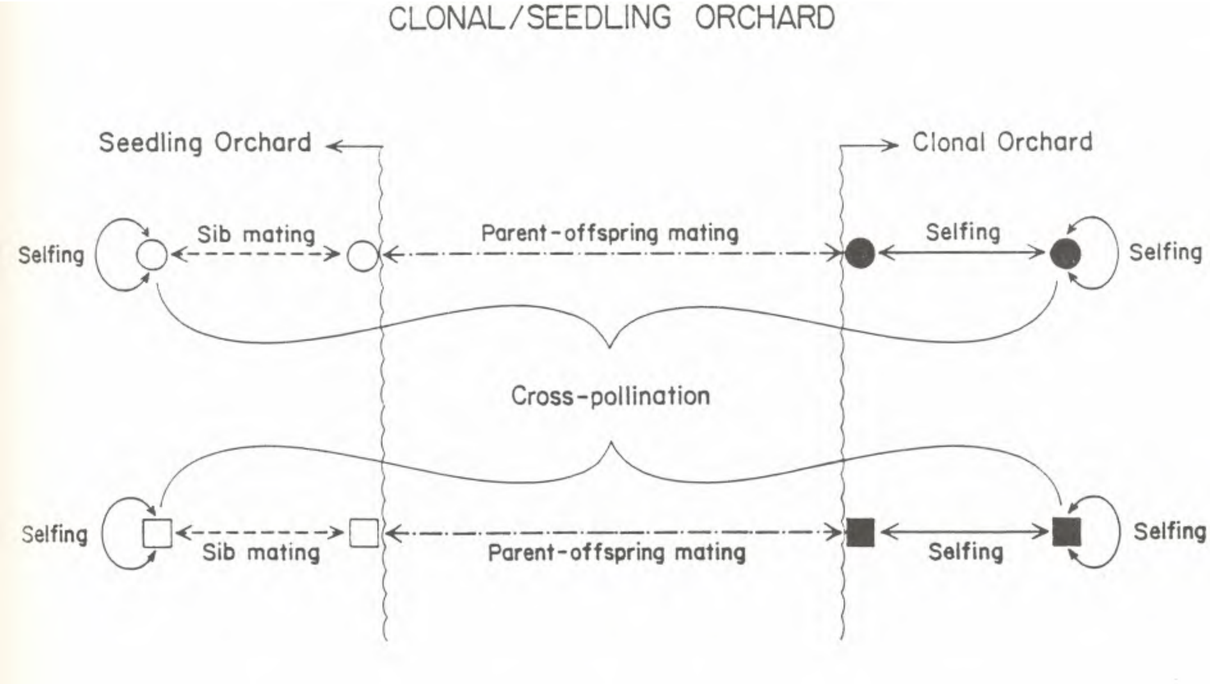

Fig. 6 Diagramatic representation of the possible mating types in clonal, seedling, and clonal/seedling orchards. ● and ■, ramets of specific clones (representing ortets ● and ■ respectively); ○ and □, open-pollinated seedlings from ortets ● and ■ respectively; ⟷, selfing; ←--→, sib mating; ←·—·→, parent-offspring mating; ⁀‿⁀, cross-pollination.

upon the fulfillment of the random-mating assumption of the Hardy-Weinberg theorem in the absence of selection, mutation and migration. Pollen migration (gene flow) provides a mechanism for maintenance of genetic variability, particularly in sessile, wind-pollinated, long-lived organisms like forest trees. The amount of gene flow (i.e., contamination) from surrounding unselected trees into seed orchards affects the genetic quality of seedlots in the following ways: 1) reduction of the expected genetic gain, 2) reduction of the within-orchard outcrossing rate, 3) production of maladapted stock and 4) change in the allelic and genotypic frequencies between the seed orchard population and its seedlots.

Methods used to reduce pollen contamination in seed orchards include: 1) geographical isolation by establishing orchards outside the species' range (Sarvas 1970; Hadders 1972) or at different elevations (Silen 1963), 2) reducing the frequency of outside pollen by the use of SMP (Woessner and Franklin 1973; Bridgwater and Trew 1981; El-Kassaby and Ritland 1986a), establishing larger orchards (Wright 1953), and surrounding the orchards by isolation zones (Wright 1953; Squillace 1967), or 3) physiological (i.e., temporal) isolation of the orchard through phenological manipulation by the use of water-spray cooling treatment (Silen and Keane 1969; Fashler and Devitt 1980; El-Kassaby and Ritland 1986b; El-Kassaby and Davidson 1989a).

The level of pollen contamination is affected by the proximity to and the size of the contamination source, reproductive synchrony between the orchard and outside source, crop size, seed orchard pollen crop size and the crop management practices used. Contamination estimates of 40 and 52% have been reported for two Douglas-fir orchards from Oregon (Smith and Adams 1983) and 44% for an orchard form Washington (Wheeler and Jach 1986). Contamination levels of 24% for early- and 18% for late-flowering trees for a Douglas-fir seed orchard from British Columbia when the cooling treatment was not used (El-Kassaby and Ritland 1986a) and a 9% for early-flowering trees when the cooling was used have been found (El-Kassaby et al. 1986a). The overall contamination level estimated for that orchard when the cooling treatment was used reached a record low of 0.2±5.7% (El-Kassaby and Ritland 1986b). The use of SMP as a contamination-preventiol method proved effective. No contamination was detected for intermediate-flowering trees that received SMP (El-Kassaby and Ritland 1986a) or for orchard blocks receiving SMP alone or in conjunction with cooling (El-Kassaby and Davidson 1989a).

Inbreeding

The mating system, the union of male and female gametes and their genetic relationship, plays a crucial part in determining subsequent population structure and on the way genetic information is being transmitted and distributed from one generation to another. Although estimates of natural outcrossing in conifers have been found to be quite high (t>0.9; Adams and Birkes 1988, for review), the level of inbreeding in seed orchards, even if it is low, is important since most forestry programs rely on non-competitive plantings in both nursery production and plantation establishment phases.

The outcrossing rates of several plant species have revealed marked differences under different environmental conditions (Clegg 1980). Orchard population structure (clonal vs. seedlings) (Ritland and El-Kassaby 1985), density (El-Kassaby et al. 1986b), phenological differences (El-Kassaby et al. 1988), contamination level (El-Kassaby and Ritland 1986a; El-Kassaby et al. 1989d) and reproductive output (Denti and Schoen 1988) all have affected outcrossing rate significantly.

In general, outcrossing-rate estimates obtained from seed orchards are higher than those reported for natural stands of the same species. This suggests that population structure (i.e., the physical arrangement of related and unrelated individuals within a population) affects the rate of outcrossing. If orchard population arrangement can increase the rate of outcrossing, then any additional manipulation of the pollination ecology could also further reduce inbreeding and, consequently, improve the seed orchard's genetic efficiency.

Cone position in the crown can influence inbreeding; higher levels of inbreeding in lower crowns of Douglas-fir orchards were reperted (Shaw and Allard 1982; Omi and Adams 1986; El-Kassaby and Davidson 1989b). However, when the overhead cooling treatment was applied, non-significant differences were reported (El-Kassaby et al. 1986b; El-Kassaby and Davidson 1989b). In addition, the overall outcrossing rate obtained from cooled orchards was higher than that obtained from uncooled ones, even under a shorter pollination season (El-Kassaby and Davidson 1989b). The shorter pollination season produced a pollen cloud of related and unrelated pollen, and early zygotic selection acted as a mechanism to weed out inbreds. In general, the clonal orchards' inbreeding level was lower than seedling ones due to the presence of only one type of inbreeding (i.e., selfing) as opposed to selfing and sib-mating in seedling orchards and parent-offspring mating of clonal/seedling orchards (Fig. 6). Reproductive phenology classes also produced different inbreeding levels: early- and late-flowering trees produced lower proportions of outcrossing than trees with more intermediate flowering periods (El-Kassaby et al. 1988).

Impact of Crop-Management Practices on the Quality and Quantity of Seed Crop

The impact of SMP and overhead cooling on the joint levels of contamination and outcrossing, and seed yield, was assessed in a 13-year-old seedling Douglas-fir seed orchard. A 2x2 factorial arrangement of SMP/no SMP and cooling/no cooling was applied to four identical blocks of trees (El-Kassaby and Davidson 1989a). The four treatment combinations studied were spatially and temporally isolated by buffer blocks and a 9-day reproductive bud phenology delay. The rate of outcrossing and contamination in the seed crop was determined from a sample of 325 trees (79-85 trees/block) that provided a total of 5,964 viable seeds (1441-1574 seeds/block). The study revealed the following conclusions: 1) no contamination was obtained when either cooling or SMP was used, 2) no inbreeding was observed when cooling and SMP were used concurrently, and 3) SMP was effective in reducing consanguineous mating, but not to the level attained form cooling and SMP combined (Table 3).

Table 3. Joint levels of outcrossing and contamination for all cooling/no cooling and SMP/no SMP treatment combinations for four identical 13-year-old Douglas-fir orchard blocks.

Treatment	Cooled		Uncooled	
	SMP	No SMP	SMP	No SMP
Outcrossing (t)	1.090±0.002	0.978±0.018*	*	0.970±0.019*
Contamination	-0.110 0.357	-0.121±0.428	-0.016±0.337	0.005±0.267

Rejection of the null hypothesis that t = 1.00 at P<0.05.
1/ Negative values indicate lack of contamination.

Seed-yield comparisons were determined form a sample of 50 cones form each of 20 trees in each treatment combination. Potential seed yield per cone, the average number of successful fertilizations and average number of filled seed per cone all showed no significant differences between cooling or SMP treatments and among cooling x SMP treatment combinations, indicated that the within-orchard pollen cloud was not a factor limiting seed yield. The average number of seeds infested by the Douglas-fir cone seed wasp (*Megastigmus spermatrophus* Wachtl) larva was significantly less when cooling was applied, indicating that the cooling treatment was effective in disrupting the synchrony between ovipositing females and developing cones (El-Kassaby et *al.* 1989a). A similar relationship was observed also by Miller (1983) for Douglas-fir cone gall midge (*Contarinia oregonensis* Foote).

The study indicted that the added benefits of SMP and/or cooling in mature Douglas-fir seed orchards are quality rather than quantity-oriented.

Genetic Gain Versus Genetic Diversity

Increasing the genetic gain in existing, tested, clonal, seed orchards could be accomplished by either genetic roguing or selective cone harvesting. Genetic roguing improves both seed and pollen parents due to the irreversible removal of low-breeding-value clones form the orchard. On the other hand, selective cone harvesting improves only the seed parent since all clones (low and high breeding value) may act as pollen donors. Genetic roguing or selective cone harvesting are possible after ranking the orchard's clones according to their breeding value. Then the acceptance level (i.e., truncation point) is determined. In most cases however, the difference between the lowest accepted and the highest rejected clones is very small, while the difference between the highest and the lowest-accepted clones is very large. It is also worth mentioning that the ranking of clones is based on breeding values determined from progeny testing of material produced by controlled crosses and not from progeny collected from the orchard following wind-pollination. Therefore, genetic roguing may have the potential to eliminate clones that could be prime receptors of a major portion of the pollen being produced from the better clones and that do not substantially differ for accepted ones (i.e., high rejected and low accepted). In other words, although being conducted for the right reasons, genetic roguing could decrease the orchard gain rather than increasing it when specific combining ability is present.

Levels of genetic diversity between a rogued orchard's seed crop and a seed crop collected from a similar orchard following selective cone harvesting also are different. In an attempt to compare the levels of genetic gain and genetic diversity present in seed crops collected from a seed orchard following genetic roguing, selective cone harvest, and a combination of the two options, Lindgren and El-Kassaby (1989) have demonstrated on theoretical grounds that combining selective cone harvest with genetic roguing is the best option available because both genetic gain and diversity were optimized. Table 4 provides examples where the "relative effective clonal number" (equivalent to diversity) is constant (0.8) under the three options (Lindgren and El-Kassaby 1989). These are: 1) when 80% of the clones were harvested (P_H) after the removal of 20% (i.e., proportion remaining P_R = 80%) ($P_H = P_R = 0.8$), 2) when 60% of the clones were harvested after the removal of 10% of the orchard clones ($P_H = 0.6$ and $P_R = 0.9$) and leaving 30% to participate in pollination, and 3) when 50% of the clones were harvested and the remaining 50% were left to participate in the pollination ($P_H = 0.5$ and $P_R = 1.0$). The results indicate that the second option (i.e., combination between selective harvest form the best clones and removal of the lower ranked clones) provided the highest genetic gain (0.419 (equivalent to effective selection intensity that is proportional to genetic gain) compared to the removal of the low-ranking clones (0.350) or the selective harvest without any removal (0.399). It should be noted that the number of trees removed from the orchard affects both the rate of inbreeding and the contamination level. Both selective cone harvest and the combination of roguing and selective harvest options provide flexibility to adjust to the genetic composition of the orchard at advanced stages in the program.

Table 4. Comparison of genetic gain and genetic diversity (upper and lower numbers, respectively) for various fractions of clones harvested (Pa) and clones remaining (P_R) after genetic roguing (source: Lindgren and El-Kassaby 1989).

P_R	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
.1	1.755	1.577	1.457	1.360	1.276	1.199	1.126	1.052	0.976	0.877
	.100	.160	.200	.226	.250	.267	.280	.291	.300	.308
.2		1.400	1.279	1.183	1.099	1.022	0.948	0.875	0.797	0.700
		.200	.267	.320	.364	.400	.431	.457	.480	.500
.3			1.159	1.062	0.978	0.901	0.828	0.754	0.677	0.579
			.300	.369	.429	.480	.525	.565	.600	.632
.4				0.966	0.882	0.805	0.731	0.658	0.580	0.483
				.400	.471	.533	.589	.640	.686	.727
.5					0.798	0.721	0.647	0.574	0.496	0.399
					.500	.571	.636	.696	.750	.800
.6						0.644	0.570	0.497	0.419	0.322
						.600	.672	.738	.800	.857
.7							0.497	0.423	0.346	0.248
							.700	.772	.840	.903
.8								0.350	0.273	0.175
								.800	.873	.941
.9									0.195	0.097
									.900	.973
1.0										0.000
										1.000

Stress and the Generation of Heritable Variation

The life cycle of plants is characterized by the presence of two distinct phases: the haploid gametophyte, which produced gametes, and the diploid sporophyte, which contains cells capable of undergoing meiosis. Shoot apical meristems produce vegetative tissue until an external signal triggers a switch to floral development. Therefore, floral structures are derived from a set of cells which are part of the vegetative body of the plant. Annuals convert most of their apicals to floral structures, while in perennials only a subset of the apices is converted from vegetative to floral development, leaving some to form vegetative buds which will support the following year's growth. The production of floral structures from vegetative parts provides a mechanism by which somatic mutations can be perpetuated through incorporation in the gene pool, perhaps increasing the overall variability of the species (Antolin and Strobeck 1985).

Stresses and shocks were considered among the major contributing factors in the appearance of these somatic variations (McClintock 1984). Durrant's (1958,1962) study on some varieties of flax is considered to be the classical example demonstrating the generation of somatic heritable variation under stressful environments (see Walbot and Cullis 1985, for review). The detection of these changed was easy in the flax study because the varieties used were stable, pure, inbred lines (i.e., maximum homozyosity and homogeneity). If shock or stress produces somatic mutation in outcrossing species, particularly in conifers, the detection of the mutation will be difficult due to the high heterozygosity and heterogenity characteristics of these species.

After working on the same flax varieties and many other plant species, Cullis (1987) indicates that the majority of the genomic alterations occur in some families of the highly repeated sequences of the DNA, and that higher plants have a genetically controlled variation-generating system that can be activatec only by shocks. He stated also that the changes occur during the growth of the plants, when inducing conditions occur before meiosis, and that these changes can be passed onto the next generation(s). Thus, single plants may consist of a mosaic of genetically different parts. Therefore, the environment has a dual role in this case: on the one hand it is the agent causing variants to arise and on the other it acts to select among these variants.

The use of stress treatments, such as cultural modification (water stress, root-pruning, stem girdling, excessive nitrogen fertilization or starvation and high temperature), hormonal application (exogenous gibberellin) or their synergistic combinations, have been proven successful for flower promotion in seed crchards (see Pharis et _al._ 1987, for review). Conifers present a suitable material for the stress-triggered mutation mechanise due to the presence of a substantial amount of highly repetitive DNA sequences in their genomes (Dhillon 1987). Stress-induced mutations have not been reported for conifers but a few reports of subtle changes are published. Changes in germinative speed caused by nitrogenous fertilization (Allen 1961) and hormonal application (Puritch et _al._ 1979) were reported for Douglas-fir, and adverse heat-related effects on fertilization and seed development were observed for _Picea engelmannii_ by Ross (1987). The use of clonal material will provide researchers with material analogous to the inbreed lines used in the flax study and changes may be observed under controlled environments. The continual use of these practices without rigorous testing is dangerous and may effectively block further inquiry.

On the other hand, physiological manipulation of reproductive phenology development by overhead cooling in Douglas-fir seed orchards did not change the observed rate of abnormal embryos and germination parameters of seed crops under cooling compared to those developed under natural conditions (El-Kassaby et al. 1989b). It was concluded that the temporal delay of reproductive phenology caused by temperature manipulation as a result of the cooling treatment is within the species' biological limits.

CONCLUSIONS

The consistent supply of genetically improved seed for reforestation programs is dependent upon the fulfillment of several conditions in seed orchards. These include reproductive synchrony and reproductive equality among the orchards' genetic entities and minimizing both inbreeding and contamination levels. The proceeding review demonstrated that these conditions are: a) not fully met, b) inter-related, and c) substantially affected by the type of crop management practice used. The use of overhead cooling and/or SMP have proven to be effective crop management tools in reducing most deviations from panmixia. Recently, most of our crop management practices have been directed towards improving the genetic quality of seed crops since our production targets have been surpassed.

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