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Impact of Crop Management Practices on Seed Yield in a Douglas-Fir Seed Orchard

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Abstract

The impact of two crop-management practices, supplemental-mass-pollination (SMP) and overhead cooling, on seed yield in a 13-year-old seedling Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seed orchard was studied. A 2 × 2 factorial trial of SMP/no SMP and cooling/no cooling was applied to four genetically similar blocks of trees. Potential seed yield per cone, average number of successful fertilizations, and average number of filled seeds per cone all showed no significant differences between cooling or SMP treatments and among cooling × SMP treatment combinations. These results indicated that the within-orchard pollen cloud was not a factor limiting seed yield. The average number of seeds infested by the Douglas-fir seed wasp (*Megastigmus spermotrophus* Wachtl) larva was significantly ($P<0.05$) less when cooling was applied, indicating that the treatment was effective in disrupting the synchrony between presence of ovipositing females and developing cones. It is concluded that the cooling was effective in reducing seed loss due to insect damage and that the added benefits of the SMP and/or cooling in mature Douglas-fir seed orchards are quality oriented rather than quantity oriented.

Key words: Douglas-fir, seed orchards, supplemental mass pollination, overhead cooling, seed potential.

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Introduction

Supplemental mass pollination (SMP), the broadcast application of viable pollen to un-isolated strobili, is a common crop-management practice in most Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seed orchards in coastal British Columbia. Since its introduction by Wakeley *et al.* (1966), the technique has been used to 1) broaden the genetic base by introducing desirable genotypes into seed orchards (Woessner and Franklin, 1973; Denison and Franklin, 1975; Hadders, 1984), 2) produce inter-specific hybrid seeds (Wakeley *et al.*, 1966; Hyun, 1969; Joly and Adams, 1983), 3) reduce self-fertilization (Woessner and Franklin, 1973; El-Kassaby and Ritland, 1986a; El-Kassaby and Davidson, 1990), pollen contamination (El-Kassaby and Ritland, 1986a; El-Kassaby and Davidson, 1990), and ovule abortion (Bridgwater and Bramlett, 1982), 4) improve parental balance (El-Kassaby *et al.*, 1986, 1989; El-Kassaby and Reynolds, 1990; Reynolds and El-Kassaby, 1990), and panmixis (El-Kassaby *et al.*, 1984, 1986, 1988; Fashler and El-Kassaby, 1987), and 5) increase seed yield (Daniels 1978; Bridgwater and Trew, 1981; Bridgwater and Bramlett, 1982; Hadders, 1984; Webber, 1987; El-Kassaby and Reynolds, 1990) and genetic gain (Denison and Franklin, 1975).

Overhead cooling of seed-orchard trees, another crop-management practice, was proposed as means of reducing orchard contamination by delaying reproductive bud development relative to background pollen sources (Silen and Keane, 1969). This procedure provides temporal isolation of pollen contamination (Fashler and Devitt, 1980; El-Kassaby and Ritland, 1986b; Fashler and El-Kassaby, 1987; El-Kassaby and Davidson, 1990) and is effective in decreasing cone and seed insect damage (MILLER, 1983). It was also observed that the cooling treatment shortened (i.e., compacted) the pollination period, which in turn increased the density of the pollen cloud inside the orchard (EL-KASSABY *et al.*, 1986; FASHLER and EL-KASSABY, 1987).

To date, 49 percent of the 57 ha first-generation Douglas-fir seed orchards in British Columbia are equipped with overhead cooling systems. In addition, a decision has been made to equip all newly established second- generation orchards (16 ha) with cooling systems. All genetically improved seeds obtained from Douglas-fir seed orchards in British Columbia are now produced under a crop-management regime that consists of SMP and/or overhead cooling.

As part of an ongoing assessment of seed orchard crop-management practices, the results of an experiment to test the effects of cooling and SMP on seed yields in four genetically similar 13-year-old, full-sib seedling orchard blocks is presented.

Materials and Methods

Seed Orchard Description

The study was conducted in the 6 ha, full-sib Douglas-fir Canadian Pacific Forest Products Limited seed orchard in Saanichton, B.C. (latitude 48°35'N; longitude 123°24'W; elevation 50 m). The seedling orchard production population consists of 110 full-sib families produced from controlled crosses made among grafts of plus-trees selected from sites ranging from 300 m to 1000 m in south- western British Columbia. Trees within the orchard's 14 blocks were planted at 4 m × 6 m spacing in 1975 as 2-year-old seedlings in a systematic design.

A special feature of this orchard is the availability of a solid-set, overhead water-sprinkling system in seven of the 14 blocks (blocks 1 to 7) (FASHLER and DEVITT, 1980). Although installed as an irrigation system, it is used also as a device to miniminze pollen contamination by delaying reproductive phenological development in the orchard.

Treatments and Experimental Design

A 2 × 2 factorial trial (cooling/no cooling and SMP/no SMP treatments) was applied during the 1986 pollination season. These four treatment combinations were separated by two buffer blocks and by a 20-m-wide space between the cooled and the uncooled sides of the orchard. In addition, the cooling treatment produced a 9-day reproductive phenology difference (temporal isolation) between the cooled and the uncooled treatments.

The SMP treatment was applied when appropriate, judged by reproductive phenology surveys conducted every second day throughout the pollination period to identify receptive trees. SMP treatment was applied to every cone-bearing tree within the six treated blocks. To account for within-tree variation, receptive trees were pollinated up to three times. SMP was applied to two crown levels (the upper crown, reached by manlift, and the lower crown, reached by ground crews). Pollen was applied to all receptive strobili using a hand-operated insecticide sprayer with a tubular wand. Pollen was pumped through the device until it was observed coming from the end of the wand. The amounts of pollen applied to receptive strobili were not strictly controlled. A total of 15 fresh pollen mixes were used. The number of pollen parents per mix ranged between 7 and 20. The volume of pollen applied was 8,880 mL.

Cone Collections

In the fall of 1986, a sample of 50 cones was collected from each of 20 trees selected at random in each treatment. Thus, sampled trees were not related. Cones were placed in burlap sacks and stored in an open-sided, freely-ventilated shed for 12 weeks prior to seed extraction. Dry cones were hand-dissected and all scales examined for developing ovules. Total seed yield was counted and average yield per cone was calculated by tree. Filled, empty, and seeds attacked by the Douglas-fir seed wasp (*Megastigmus spermotrophus* WACHTL) were determined using X-ray methods.

Seed loss caused by the Douglas-fir cone gall midge (*Contarinia oregonensis* FOOTE) was not considered in

Table 1. — Effect of overhead cooling and supplemental-mass-pollination (SMP) on potential number of seeds per cone (PNSC), successful number of fertilizations per cone (SNFC), number of filled seeds per cone (NFSC), and number of seeds with larva per cone (NSLC) for seeds collected from a 13-year-old seedling Douglas-fir seed orchard.

Source of Variation	d.f.	E.M.S.[1/]	PNSC		SNFC		NFSC		NSLC	
			MS	P	MS	P	MS	P	MS	P
Cooling (C)	1	$\sigma_e^2 + K_1\phi_c$	9.47	0.812	252.22	0.112	286.41	0.089	0.902	0.015
SMP	1	$\sigma_e^2 + K_2\phi_{smp}$	575.88	0.066	330.09	0.070	350.31	0.060	0.248	0.193
C x SMP	1	$\sigma_e^2 + K_3\phi_{csmp}$	4.86	0.864	58.09	0.442	54.20	0.455	0.066	0.502
Residual	75	σ_e^2	165.52		97.43		96.17		0.144	

1/ σ_e^2 = variance due to differences among trees within cooling and SMP treatment combinations:
ϕ_c = variance due to cooling treatment;
ϕ_{smp} = variance due to the SMP treatment;
ϕ_{csmp} = variance due to the interaction between cooling and SMP; and
K_1—K_3 = are the coefficients of variance components.

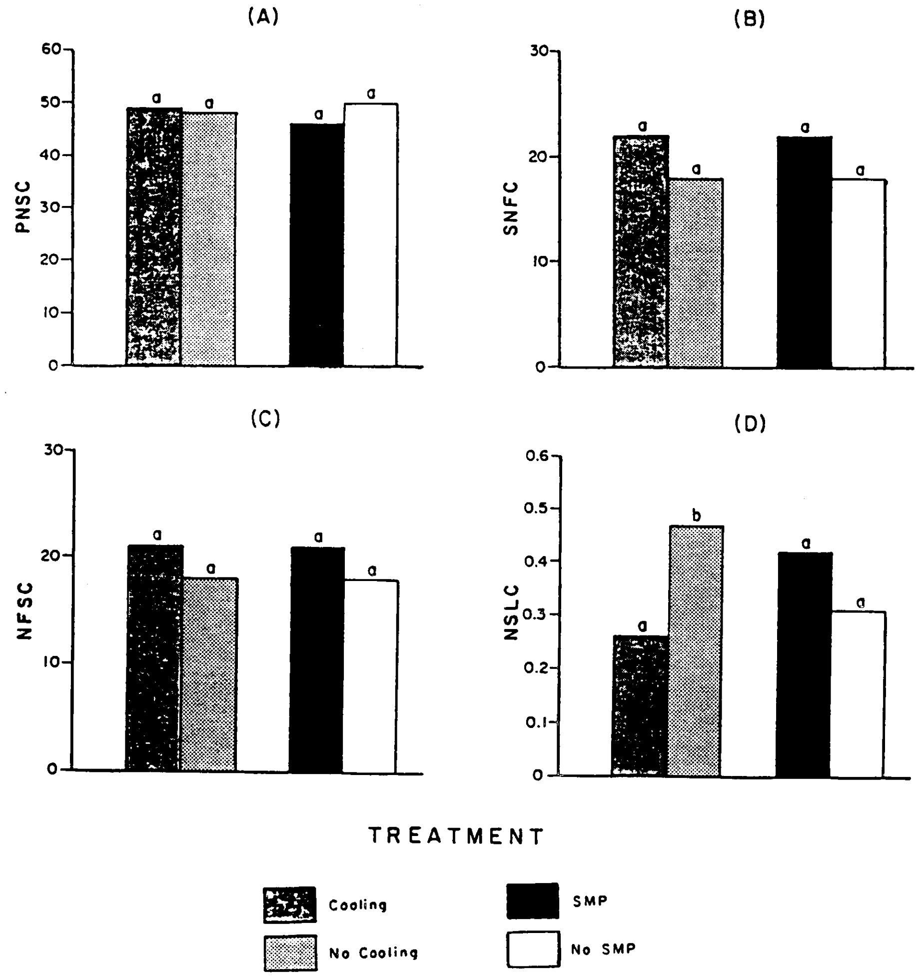

Figure 1. — Comparison between cooling and supplemental-mass-pollination (SMP) on (A) potential number of seeds per cone (PNSC), (B) successful number of fertilizations per cone (SNFC), (C) number of filled seeds per cone (NFSC) and (D) number of seeds with larva per cone (NSLC) for seeds collected from a 13-year-old seedling Douglas-fir seed orchard. (Bars having the same letter are not significantly different, $P < 0.05$).

this study. This was based on results from two samples of 100 conelets collected from the cooled and uncooled parts of the orchard. The examination of these samples indicated that the level of infestiation by the Douglas-fir cone gall midge was below the established threshold at which significant seed loss could be expected and that no significant difference was observed between the cooled and uncooled parts of the orchard (D. Summers, Silviculture Branch, B. C. Ministry of Forests, letter, May 23, 1986).

Statistical Analysis

The average number of seeds per cone, number of filled seeds per cone ,and number of seeds with insects per cone were subjected to a standard two-way analysis of variance. The ANOVA was based on the following additive linear model:

$$Y_{ijk} = \mu + C_i + SMP_j + C.SMP_{ij} + \varepsilon_{(ij)k}$$

where Y_{ijk} is the value of the kth tree (random effect) in the jth SMP treatment in the ith cooling treatment, μ the general mean, C_i the effect due to the ith cooling treatment (fixed effect), SMP_j the effect due to the jth SMP treatment (fixed effect), $C.SMP_{ij}$ the effect due to the ith cooling × the jth SMP treatment interaction, and $\varepsilon_{(ij)k}$ the residual error term.

Results and Discussion

The cooling treatment produced a 9-day temporal isolation between the cooled and the uncooled parts of the orchard, in addition to the spatial separation imposed by the buffer blocks between the SMP/no SMP sections and the 20-m-space between the cooling/no cooling treatments. The delay in reproductive phenology and the resultant shorter breeding season in the cooled part, has been proven to be effective in both reducing the contamination rate and increasing the cross-pollination level (El-Kassaby and Davidson, 1990).

A comparison of the average potential seed yield per cone (estimated as 2 × the scales bearing fully developed ovules) was conducted among the four treatments. No significant difference in average potential seed yield per cone was observed for cooling, SMP, or cooling × SMP interaction (*Table 1, Figure 1A*), indicating that cone size variation was not a factor in the results.

Average number of successful fertilizations per cone by parent was determined by adding the number of filled seeds to those seeds containing *M. spermotrophus* larvae and dividing the total by the number of cones sampled. Douglas-fir seed wasps lay eggs in the seeds of immature cones in June (Ruth, 1980); the female oviposits them through the cone scale and into the developing seeds. The small larva feeds on the tissues within the seed, leaving only the seed coat. Feeding is completed in 6 to 8 weeks, and the larva overwinters in the seeds. Eggs laid in unfertilized seeds produce no larvae (HUSSEY, 1956). Thus, the presence of a larva inside a seed is an indication of successful fertilization even though a filled seed no longer exists (D.S. RUTH, Forestry Canada, Pacific Forestry Centre, Victoria, B.C., pers. comm., Jan. 1989). Derivation of the average number of successful fertilizations per cone was necessary to compare the effect of cooling and SMP treatments. It must be emphasized that this study was conducted on an operational scale to produce a realistic assessment of these management practices. Insect damage was an important consideration in this study because the trial was not conducted on isolated, bagged cones.

No significant differences were found in the average number of successful fertilizations and filled seeds per cone for cooling, SMP, and cooling × SMP interaction effects (*Table 1, Figure 1 B to C*). The data from the present study are constant with those from a 16-year-old Douglas-fir seed orchard (DANIELS, 1978) and the mid- and late-flowering trees in another 10-year-old Douglas-fir seed orchard (WEBBER, 1987).

SMP increases seed yield only when pollen production within the orchard or contamination is limited. However, the production of a dense pollen cloud and low seed yield in mature orchards has been observed when the development of male and female strobili is out of synchrony (EL-KASSABY and REYNOLDS, 1990) or when severe insect infestations occur (BRAMLETT, 1987). As in the present study, BRIDGWATER and BRAMLETT (1982) confirmed that SMP did not increase seed yield in a mature loblolly pine (*Pinus taeda* L.) seed orchard, but a significant increase was observed in a newly established (i.e., immature) orchard. EL-KASSABY and REYNOLDS (1990) reported that SMP increased seed yield 14-fold in a mature Sitka spruce [*Picea sitchensis* (BONG.) CARR.] orchard, the lack of reproductive male and female synchrony being the sole reason for that increase.

In the present study, the within-orchard pollen production apparently was dense enough to service all receptive sites; the increase in the available pollen provided by either the SMP and/or cooling (through the shortening of the pollination period in the latter treatment) did not increase filled-seed yield. A comparison of seed set between wind-pollinated cones from the control (no SMP, no cooling) treatment and the other three treatments is not valid as an expression of seed orchard efficacy unless the level of contamination by pollen from outside the orchard is known. The level of contamination for the four treatments, estimated using isozyme markers, was very low in the control treatment and virtually nonexistant in all other treatments (EL-KASSABY and DAVIDSON, 1990). Thus, the observed lack of significant differences among the cooling/no cooling and SMP/no SMP treatments in this study was interpreted to indicate the presence of adequate, effective, pollen within the orchard.

The difference in the average number of seeds with *M. spermotrophus* larva was significant ($P < 0.05$) between the cooling/no cooling treatment (*Table 1, Figure 1D*). This was likely because the delay in strobilus production caused by the cooling treatment disrupted the synchrony between the presence of ovipositing female wasps and developing cones in the orchard. On the other hand, no significant difference was observed between SMP/no SMP treatment (*Table 1, Figure 1D*). This was expected since the SMP treatment did not affect the insect population behaviour. HUSSEY (1955) reported that the synchrony between presence of wasps and susceptible host stage is sensitive to weather changes. A similar relationship was observed by MILLER (1983) for Douglas-fir cone gall midge (*Contarinia oregonensis* FOOTE). The reproductive bud delay caused by the cooling treatment in this study seems to have upset this relationship, resulting in a significant reduction in the *Megastigmus* infestation level.

The presence of *M. spermotrophus* larva in Douglas-fir seeds is problematical. Current seed extraction/separation methods isolate infested seeds with difficulty. This will cause an underestimation of the germinability since the "real" germination, based on non-infested, filled seeds, will be greater than the "apparent" germination for all seeds in the mixture. Consequently, nursery sowing factors require that several seeds be sown to ensure at least one germinant per cavity. Removal (thinning) of supernumerary seedlings is time consuming and very expensive. Even though seed yields from this orchard have reached the point of supplying seeds in excess of local requirements, such multiple sowing is wasteful of seed resources. In addition, moisture contents of infested seedlots are often overestimated when there is a significant number of larvae in the seedlot; empty seeds (seeds devoid of embryo or megagametophytic tissues) and those containing larvae typically are higher in moisture content (on a weight basis) than completely developed, filled seeds (EDWARDS, unpub. data). Adjustment of moisture content of these seedlots for cold storage will therefore tend to over-dry the filled seeds. A reliable method of separating infested seeds from filled seeds is needed. However, the reduction in insect attack by overhead cooling will simplify seed extraction and storage, and reduce nursery costs.

In conclusion, this study confirmed previous observations on the effect of the overhead-spray cooling treatment in reducing seed loss due to insect damage, and extended the effects to *M. spermotrophus*. The benefits of SMP and/or cooling in mature Douglas-fir seed orchards appear to be in improving seed-quality rather than seed-quantity.

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Impact of Crop Management Practices on the Seed Crop Genetic Quality in a Douglas-fir Seed Orchard

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Abstract

The impact of two crop management practices, supplemental-mass-pollination (SMP) and overhead cooling, on levels of pollen contamination and outcrossing was assessed in a 13-year-old seedling Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seed orchard with the aid of six allozyme loci. A 2 × 2 factorial arrangement of SMP/no SMP and cooling/no cooling was applied to four genetically similar blocks of trees. The four treatment combinations used were spatially and temporally isolated by buffer blocks and nine-day reproductive bud phenology delay, respectively. The study revealed the following conclusions: 1) no contamination was obtained when cooling and/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 from cooling and SMP combined. In addition, the results obtained from the control block (i.e., no cooling and no SMP) indicate: 1) the effectiveness of isolation zones in reducing contamination and 2) randomization of trees within seed orchard blocks is associated with a high outcrossing rate.

Key words: Douglas-fir, seed orchards, supplemental-mass-pollination, overhead cooling, contamination, outcrossing rate.

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Introduction

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. Forest trees, conifers in particular, are wind-pollinated and have been characterized as predominantly outcrossing ($\underline{t} > 0.9$: Adams and Birkes, 1990, for review), among the most genetically heterozygous of plants (Mitton, 1983), and often displaying a significant level of inbreeding depression (Franklin, 1970).

To date, seed orchards are becoming an important source for the production of seed for most of the economically important conifer species' reforestation programs. These programs rely on non-competitive plantings in both seedling-production (i.e., nursery) and plantation-establishment phases. The presence of inbred or mildly inbred seeds or seedlings produced by inbreeding (selfing or consanguineous matings) may survive past the nursery stage, but likely would suffer from inbreeding depression, which may lead to inferior growth and vigour as mature trees.

In order to maximize the genetic efficiency (i.e., high genetic quality and broad genetic variability) in wind-pollinated seed orchards, forest geneticists rely on several methods to control the mating system of orchard trees.