is at a low density in surrounding areas. Much of the current population may be descendants of only a few trees that survived the original fire at this particular site. It is even possible that more than one inbred line may be represented in the population.

On the other hand, additional evidence does not support high levels of inbreeding. Trees found to be heterozygous for the albino allele were growing in close proximity. Wind-pollinated progenies from most trees did not show obvious inbred characteristics. Performance of progenies of this population in two other tests (REHFELDT 1974 a, 1974 b) was normal. And the performance of progenies developed from interpopulational crosses that included the present population did not differ from the mean performance of intrapopulational crosses of parental populations (G. E. REHFELDT, unpublished data). Therefore, either the percentage of inbred trees (12%) within the present population is overestimated, or a similar level of inbreeding is characteristic of the genetic structure of all populations. The latter alternative may be of particular importance. Douglas-fir is a seral species on the Tsuga heterophylla series of habitats of northern Idaho, a region in which wild fire has been the primary agent of forest disturbance (DAUBENMIRE and DAUBENMIRE 1968).

The level of inbreeding within the population should be expressed in the frequency of numerous lethal or semilethal traits. Unfortunately, the objectives for which this study was designed did not include an assessment of inbreeding within the species' mating system. Consequently, data on seed development and the oecurrence of other mutant phenotypes were not obtained.

Still, results of this study have implications for studies in ecological genetics and for tree improvement of Rocky Mountain Douglas-fir. No differences in mean performance or variation of performance could be associated with wind- or local cross-pollinated seeds. For species of similar genetic systems, wind-pollinated seeds seem to adequately reflect population structure. Secondly, even for Douglas-fir, a species characterized by high inbreeding depressions and relatively large genetic loads, inbreeding may be a constituent of the mating system. Even though inbreeding could account for random patterns of local differentiation (REHFELDT 1974 a), the importance of inbreeding on the evolution of the genetic system requires further assessment.

That inbreeding inflates additive genetic variances is well documented. For Rocky Mountain Douglas-fir, genetic components of variance and genetic gains can be estirnated only after assessing the importance of inbreeding on the genetic structure of populations.

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Efficacy of supplemental mass-pollination in a Douglas-fir seed orchard

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Summary

A study of eight different pollination regimes, encompassing a common ten-day period in a 16-year-old Douglas-fir orchard, quantified day-to-day effects of wind pollination, controlled pollination, and supplemental mass-pollination on filled seed production of a single clone. Wind pollination had a marked cumulative effect, with each additional day of open pollination resulting in an average increase in filled seed percent (FS%) of eight percentage points. Wind-pollinated controls averaged 59.5% filled seed with 22.5 filled seeds per cone.

Control-pollinated treatments exhibited a temporal pattern of FS%, reflecting female strobilus receptivity, that peaked at the level of the wind-pollinated control and declined sharply between the seventh and eighth days. This rapid loss of receptivity was not signalled by any gross change in outward appearance of female strobili. Onset of female receptivity was not clearly defined due to a putative "carryover pollination" effect.

Supplemental mass-pollination treatments (i.e., a single application of pollen to unbagged strobili) did not appreciably increase FS% above the mean of the wind-pollinated control, in this particular orchard clone. However, analysis of other pollination regimes indicated that supplemental mass-pollination could significantly increase filled seed production in very early or late-flowering clones or in young orchards where wind pollination is more inhibited than in this study.

Proper timing of pollen application is the key to maximizing effects of supplemental mass-pollination. Special equipment and procedures are needed to facilitate proper timing and repeated applications, while conserving the costly pollen supply. Each orchardist must assess the practicality of supplemental mass-pollination, carefully weighing anticipated benefits against associated costs.

Key words: Douglas-fir, *Pseudotsuga menziesii*, mass-pollination, wind pollination, control-pollination, pollen management.

Zusammenfassung

In einer Samenplantage mit 16 Jahre alten Douglasien-Pfropflingen wurde während einer 10 tägigen Periode innerhalb der Blütezeit untersucht, inwieweit sich bei den einzelnen Klonen durch zusätzliche Bestäubungsmaßnahmen der Anteil befruchteter Samen erhöhen läßt. Frei abgeblühte Klone steigerten den Anteil befruchteter Samen etwa um 8% pro Tag und erreichten insgesamt 59,5%. Durch zusätzlich auf die weibliche Blüte gebrachten Pollen erhöhte sich der Anteil befruchteter Samen nur bei extrem früh oder spät blühenden Klonen.

Introduction

Pollen supply is a potential limiting factor in conventional forest-tree seed orchards, designed and managed for production of wind-pollinated seed. Orchard seed production — its quantity and genetic value — is contingent upon adequate cross-pollination among orchard trees. In young orchards, pollen production is usually too scanty to assure adequate pollination of the seed cones produced. This usually results in either low seed yields or high percentages of seed of dubious genetic value — depending on the degree of orchard isolation from contaminating foreign pollen.

Even in more mature orchards, seed production potential is jeopardized by the flowering idiosyncrasies of orchard trees and the vagaries of wind-pollination. Cross-pollination is hindered by sporadic and non-synchronous flowering of orchard clones, with resultant reductions in seed yields and genetic gains. Under such conditions, losses often are compounded by increased levels of selfing in clones with synchronous male and female flowering patterns (Hadders and Koski, 1975).

Supplemental mass-pollination (i.e. application of pollen to unbagged orchard flowers for commercial production of seed) is a promising alternative to total reliance on natural, wind-pollination in seed orchards. Denison and Franklin (1975) summarized the benefits of supplemental mass-pollination and reviewed the practical applications of the technique. All of the cases cited dealt with species of *Pinus;* apparently, there is a dearth of published information for other commercially important conifers.

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, is a species for which supplemental mass-pollination technology, if developed, would undoubtedly find wide application. Stettler and Cummings (1973) listed 43 Douglas-fir seed orchards operated by some 21 different organizations in the western United States and Canada. These comprise, in aggregate, no less than 180 hectares (Feilburg and Søegaard, 1975).

Some Douglas-fir orchardists, seeking ways to increase seed production, have tried various supplemental mass-pollination techniques on a limited scale, with varying results. For example, in a trial conducted in a 7-year-old grafted orchard on Vancouver Island, mass-pollinated cones yielded almost six times as many filled seeds per cone (18.7) as the wind-pollinated controls (3.3) (M. Crown, unpublished data). In contrast, a trial in a 16-year-old grafted orchard in western Washington indicated that filled-seed yields of mass-pollinated cones were no higher than those from wind-pollinated controls (15.1 vs 14.5) (S. C. Cade, unpublished data). Such discrepant examples merely serve to emphasize the need for more thorough investigation in order to quantify the benefits that might be realized from supplemental mass-pollination in various orchard situations.

A Supplemental Mass-pollination Study

In 1973, a study was designed to evaluate the efficacy of supplemental mass-pollination in Douglas-fir seed orchards. It was conducted in Weyerhaeuser Company's McDonald Orchard, located near Boistfort (Lewis County), Washington. At the time of the study, the orchard, comprising some 2.5 hectares, contained 22 clones, with 426 grafted ramts varying in age from 8 to 16 years and ranging in height from 5 to 10 meters. All of the clones had some history of flowering during the two years preceding the study. In 1973, flowering was generally good among all the orchard clones. All ramets produced at least a few female strobili, and 73 percent of the ramets bore crops that were classified as "medium to heavy". Male strobili were produced on 97 percent of the orchard ramets, and pollen production was medium to heavy on 62 percent of the ramets.

Materials and Methods

One orchard clone was chosen as the experimental seed parent; the size and complexity of the design precluded clonal replication in this initial study. Clone 2809 was selected for its heavy strobilus production and a flowering period somewhat intermediate in the array of orchard clones. Twenty healthy, **16-year-old** ramets of clone 2809 were selected as study trees.

The experimental design comprised seven different treatment regimes spanning a common 10-day period and constituting 70 individual treatments (i.e. 7 pollination modes × 10 days application). The seven regimes are described below and shown graphically in *Figure 1.*

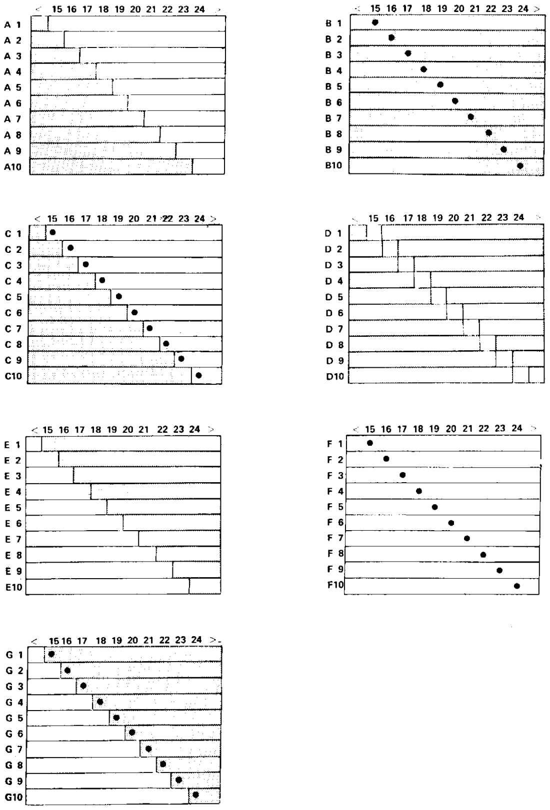

Fig. 1. — Representaiton of pollination regimes illustrating dates in April when female strobili of Douglas-fir were either bagged (shaded) or not bagged (unshaded). Dates of supplemental pollination are also shown (•).

Treatment Regime	Mode of Pollination
A.	All ♀ 's were bagged at outset; 10 percent of ♀ 's were unbagged each day of the 10-day period; no artificial pollination was applied.
B.	All ♀ 's were bagged at outset and throughout the 10-day period; 10 percent of ♀ 's were artificially pollinated each day.
C.	All ♀ 's were bagged at outset; 10 percent of ♀ 's were artificially pollinated each day, and the bags were removed immediately after pollination.
D.	All ♀ 's were bagged at outset; 10 percent of ♀ 's were unbagged one day and rebagged the following day; no artificial pollination was applied.
E.	All ♀ 's were exposed (i.e. not bagged) at outset; 10 percent of ♀ 's were bagged each day; no artificial pollination was applied.
F.	All ♀ 's were exposed at outset and throughout experiment; 10 percent of ♀ 's were artificially pollinated each day.
G.	All ♀ 's were exposed at outset; 10 percent of ♀ 's were bagged each day and artificially pollinated immediately after bagging.

Each of the 70 treatments was replicated 10 times; i.e. it was applied to each of 10 ramets of the clone scattered randomly through the orchard. One to six female strobili on each ramet constituted a treatment unit (i.e. a plot). These strobili were situated near the top of the tree to reduce occurrence of self-pollination.

For those treatments requiring artificial pollination, fresh pollen lots, collected from five clones in the orchard, were mixed in equal proportions (by volume) and applied to female strobili (both bagged and unbagged) via a hypodermic syringe fitted with a rubber squeeze-bulb. Each strobilus was sprayed repeatedly to assure pollination.

Observations of female strobilus development were made for each plot, using the following coded classifications:

Stage #	Description
1	bud swelling
2	strobilus < ¼ exposed
3	strobilus ¼ to ½ exposed
4	strobilus ½ to ¾ exposed
5	strobilus ¾ to fully exposed
6	bracts reflexed
7	strobilus pendant

Female strobili for A, B, C, and D treatments were initially bagged in stage #1, using "Terylene" bags with windows to permit observation of flowering stages. Bagging for E and G treatments proceeded as shown in *Figure 1*. All bags were finally removed when strobili were pendant (stage #7). A one-percent Lindane spray was applied to all strobili at the time of debagging as protection against cone and seed insects.

In the fall, surviving cones in each plot were collected for processing as individual seedlots. From each of the 20 study trees, five open-pollinated cones were collected to serve as a control (treatment X).

After air-drying, cones were torn apart manually in order to recover every seed. Extracted seedlots were counted and analyzed, using X-ray radiographs. For each seedlot, the following data were recorded:

- number of cones collected
- total number of seeds extracted
- number of good seeds (i.e. normal embryo and endosperm)
- number of abnormal seeds (i.e. abnormal embryo and/or endosperm)
- number of empty seeds (i.e. hollow seed coat)
- number of insect-infested seeds (i.e. containing larva of Douglas-fir seed chalcid, *Megastigmus spermotrophus* Wachtl).

For the purposes of this study, good, abnormal, and insect-infested seed data were pooled as "filled seeds", under the assumption that all represented successful pollination and fertilization.

From these basic data, the following quantities were derived:

- total number of seeds per cone (TSC)
- number of filled seeeds per cone (FSC)
- number of filled seeds per cone (FSC)

These three variables were used in statistical analyses to assess treatment effects.

Data Analysis and Results

Missing Data

The original design of this experiment included 10 replicates of each of the 70 treatments (700 plots). Eighty-eight (13 percent) of the planned plots were lost as a result of strobilus mortality and other factors (e.g. damaged pollination bags, broken branches). These losses were not unusual and were not statistically related to treatments. The number of replicates remaining per treatment ranged from six to 10, with 65 (93 percent) of the 70 treatments retaining eight or more replicates.

Total Seeds per Cone

Table 1 summarizes pertinent statistics for total seeds per cone (TSC) data from the 612 surviving plots. The overall mean for the experiment was 27.4 seeds per cone, with a standard error of 0.6. This is a somewhat lower mean and a much larger variance than was expected for this trait. Based on past experience, it was presumed that the clonal mean TSC would fall between 40 and 70 and that individual plot values would vary only slightly in this experiment, since only a single seed-clone was used. Furthermore, pollination treatments were not expected to greatly affect TSC values because, in Douglas-fir, formation of empty seeds is very common and may result from pollination with non-viable pollen, a lack of pollen during female receptivity, or self-incompatibility (Allen and Owens, 1972).

In actuality, 23 (4 percent) of the 612 plots produced no seed at all and 92 (15 percent) yielded less than 10 seeds per cone. Approximate F-tests, based on least squares analysis of variance adjusted for missing plots revealed treatment regimes to be a highly significant ($\alpha = .01$) source of variation in TSC; whereas day-to-day variation within regimes was not statistically significant.[1]

One possible explanation for the unusually low and highly variable TSC values in this experiment is unidentified insect damage. A number of different insects can cause severe seed losses in Douglas-fir cones, and each is capable of destroying a high percentage of the seed crop (Johnson and Hedlin, 1967). One such insect is the Douglas-fir cone midge (*Contarinia oregonensis* Foote). Adults are active during the spring, laying eggs in developing female strobili about the time of pollination. Larvae tunnel into ovuliferous scales causing gall formation which prevents seed development or fuses the seed and cone scale. This

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[1] In this paper, the following conventions are used to express statistical significance:
$\alpha < .05$, non-significant (ns)
$\alpha = .05$, significant (*)
$\alpha = .01$, highly significant (**)
$\alpha > .0.001$, very highly significant (***)

Table 1. — **Summary of Total Seeds per Cone (TSC) Statistics for Pollination Treatments, Regimes and Control**

Treatment	reps	mean	S.E.
A 1	9	30.3	4.2
2	10	23.5	2.7
3	9	16.0	3.6
4	9	23.9	3.5
5	9	18.1	4.7
6	9	24.0	3.9
7	9	22.9	4.2
8	8	19.6	3.2
9	8	28.1	6.0
10	9	29.9	4.6
Regime A	89	23.6	1.3
B 1	8	21.4	6.4
2	10	26.6	5.2
3	8	36.2	5.6
4	8	25.1	6.1
5	8	30.8	6.4
6	10	28.5	4.9
7	7	34.8	6.8
8	8	34.0	4.7
9	9	36.4	8.7
10	9	24.9	4.3
Regime B	85	29.7	1.9
C 1	9	10.0	2.3
2	9	19.4	4.0
3	9	23.4	3.5
4	9	20.0	2.7
5	8	20.9	5.0
6	10	17.5	3.3
7	9	24.1	2.7
8	9	22.7	5.1
9	9	27.4	4.4
10	8	23.8	5.2
Regime C	89	20.8	1.3
D 1	8	24.8	5.4
2	6	22.5	2.6
3	8	33.0	4.7
4	9	25.9	6.0
5	6	36.2	4.2
6	8	32.0	4.8
7	9	17.3	4.4
8	9	32.9	4.9
9	6	31.4	4.4
10	9	20.7	5.3
Regime D	78	26.9	1.6
E 1	9	26.6	7.0
2	8	32.8	7.7
3	9	31.7	8.6
4	10	30.1	5.0
5	9	41.0	5.1
6	9	38.7	3.3
7	9	37.7	7.3
8	8	31.5	7.5
9	10	33.9	5.0
10	9	24.3	2.3
Regime E	90	32.8	1.9
F 1	10	29.3	5.1
2	10	23.8	6.7
3	8	20.6	7.8
4	10	28.5	4.9
5	9	27.6	5.1
6	10	28.5	6.4
7	9	25.3	4.2
8	9	33.0	5.3
9	7	29.8	3.0
10	9	28.7	6.0
Regime F	91	27.8	1.7
G 1	8	28.6	4.0
2	10	32.7	6.5
3	8	36.4	8.8
4	10	23.7	7.1
5	10	35.6	4.9
6	8	27.0	7.3
7	10	34.4	5.8
8	9	26.0	4.4
9	9	27.3	5.4
10	8	28.9	6.6
Regime G	90	30.1	1.9

Control	reps	mean	S.E.
X	19	36.0	2.1

Regimes	reps	mean	S.E.
A-G	612	27.4	0.6

type of damage could result in low TSC values and might go unnoticed by seed processing personnel who were not trained to detect it.

If this were the case, one might expect to find significant differences in TSC values for cones that were bagged vs. unbagged during the period of cone midge attack (i.e. pollen flight period). However, no significant difference was found between means of bagged treatments, B1 through B10 (TSC = 29.7 ± 3.8) and unbagged treatments, F1 through F10 (TSC = 27.5 ± 3.4). In a later study, cone midge damage was actually observed in control-pollinated strobili shortly after pollination, verifying that bagged strobili are indeed susceptible to attack.

It should be noted that the open-pollinated control (Treatment X, *Table 1)* has the largest mean TSC value and smallest variance of all treatment regimes. This is probably the result of biased sampling, since control plots were not designated at the outset of the experiment. A specified number of open-pollinated cones were merely collected from each of the replicate trees at the time of harvest. It is quite likely that the pickers selected healthy, well-formed cones when they were available, thus discriminating against insect-infested cones with lower TSC values.

Filled Seeds per Cone and Filled Seed Percent

An attempt was made to reduce the confounding effects of insect depredations by rejecting all plots which averaged less than 20 total seeds per cone. A total of 404 plots were retained, and these purged data were used in all subsequent analyses. Because of these data problems, filled seed per-

Table 2. — **Summary of Filled Seed Percent (FS%) and Filled Seeds per Cone (FSC) Statistics for Pollination Treatments, Regimes and Control (Excluding Plots with <20 Total Seeds per Cone)**

Treatment	reps	FS% mean	FS% S.E.	FS% test[1]	FSC mean	FSC S.E.
A 1	7	80.7	2.7	a	28.9	2.7
2	6	74.8	3.9	ab	21.7	1.9
3	2	73.5	2.5	ab	22.5	6.5
4	7	67.6	7.8	b	19.0	3.0
5	4	64.5	10.2	b	20.8	5.3
6	7	43.4	7.0	c	12.7	2.2
7	6	46.0	5.6	c	15.7	2.2
8	5	24.6	9.3	d	6.2	2.5
9	6	24.5	10.6	d	8.7	4.6
10	7	8.1	4.3	e	3.3	1.7
Regime A	57	49.6	3.9		15.5	2.4
C 1	0	-	-	-	-	-
2	5	72.6	4.7	a	20.8	3.2
3	6	72.3	2.0	a	21.8	2.1
4	5	77.2	5.0	a	20.0	2.2
5	3	73.0	9.6	a	26.7	7.2
6	4	65.0	8.2	a	18.8	4.0
7	6	67.0	6.8	a	20.0	3.1
8	5	50.0	9.8	b	17.8	4.1
9	6	14.0	7.6	c	4.0	1.9
10	5	29.6	13.4	d	11.2	6.4
Regime C	45	56.6	4.0		17.3	1.5
E 1	5	18.0	12.6	a	9.8	7.2
2	6	35.0	10.7	ab	16.2	5.6
3	5	52.4	18.2	bc	29.8	11.9
4	7	54.3	6.8	bc	21.6	3.0
5	9	65.8	6.5	bc	29.8	5.5
6	9	64.0	4.9	bc	24.4	2.8
7	7	66.7	5.7	c	31.3	3.2
8	6	66.0	8.9	bc	27.8	6.9
9	8	48.4	6.8	bc	20.6	4.2
10	7	64.4	4.7	bc	17.6	2.1
Regime E	69	55.2	3.0		23.3	1.7
G 1	7	61.6	10.5	a	19.4	3.5
2	7	63.3	10.3	a	25.6	4.8
3	5	67.2	7.8	ab	36.2	3.6
4	6	73.5	6.1	b	27.8	6.5
5	8	77.2	4.2	b	32.2	4.2
6	5	57.8	9.4	a	21.8	4.0
7	7	79.7	4.0	b	33.4	4.0
8	6	68.7	5.4	ab	23.5	3.4
9	6	66.3	7.1	ab	23.8	5.4
10	4	60.2	11.3	a	27.8	5.6
Regime G	61	68.3	2.4		27.2	1.5
B 1	4	24.5	6.2	a	9.3	3.3
2	6	48.7	13.7	bcd	17.5	5.1
3	8	44.9	11.5	bc	17.0	4.5
4	4	57.8	13.1	cd	24.8	6.6
5	6	47.7	12.2	bcd	20.0	6.6
6	6	65.0	12.4	d	26.5	6.6
7	5	52.2	13.6	cd	21.4	3.9
8	7	32.1	13.8	ab	14.0	6.1
9	6	28.5	9.4	a	17.0	7.6
10	6	33.2	14.1	ab	11.5	4.9
Regime B	58	43.3	4.0		23.3	1.7
D 1	5	55.8	5.8	a	19.4	3.6
2	3	14.7	10.9	e	3.7	2.7
3	7	50.7	6.7	a	18.1	3.0
4	7	52.7	7.2	a	18.0	4.0
5	6	21.8	7.4	de	7.7	3.0
6	6	48.5	8.9	ab	18.5	3.5
7	3	37.3	13.9	bc	13.3	5.6
8	7	23.3	5.8	de	9.0	2.0
9	5	29.8	8.9	cd	3.8	1.5
10	4	16.8	3.1	e	4.5	1.6
Regime D	53	37.0	3.0		12.4	1.3
F 1	7	62.7	3.9	abc	22.1	5.0
2	4	63.0	9.9	abc	28.8	11.5
3	3	58.3	12.7	ab	28.3	11.6
4	7	68.3	4.8	abc	25.9	3.0
5	7	62.1	6.0	abc	22.0	4.9
6	7	64.0	6.4	abc	26.4	6.0
7	7	73.0	9.5	bc	22.4	3.8
8	7	62.0	8.9	abc	23.9	4.5
9	7	54.8	7.1	a	17.3	3.2
10	5	75.8	4.7	c	31.2	6.6
Regime F	61	64.5	2.3		24.2	1.6
A-G	404	53.9	1.3		20.0	0.6
Control X	18	59.5	3.2		22.5	1.5

[1] Treatment means with one or more common letter designators do not differ significantly, according to Duncan's Multiple Range Test (α = .05).

cent (FS%) was chosen over filled seeds per cone (FSC) as the more reliable response variable; the latter, being more strongly correlated (r = 0.54***) with "total seeds per cone" (TSC) than the former (r = 0.03 ^{ns}), would be more sensitive to TSC perturbations caused by insects.

Table 2 summarizes the pertinent statistics for both FS% and FSC for all pollination treatments, regimes and control. However, only FS% will be included in the following discussion.

Least squares analysis of variance revealed FS% variation among regimes and among treatments within regimes to be very highly significant. Duncan's multiple range test method for groups with unequal replication (Steele and Torrie, 1960) was used to make comparisons among means of the eight treatment regimes (including control) and among means of treatments within individual regimes.

Comparison of Regime Means

Results of the range test of regime means are shown below[2]):

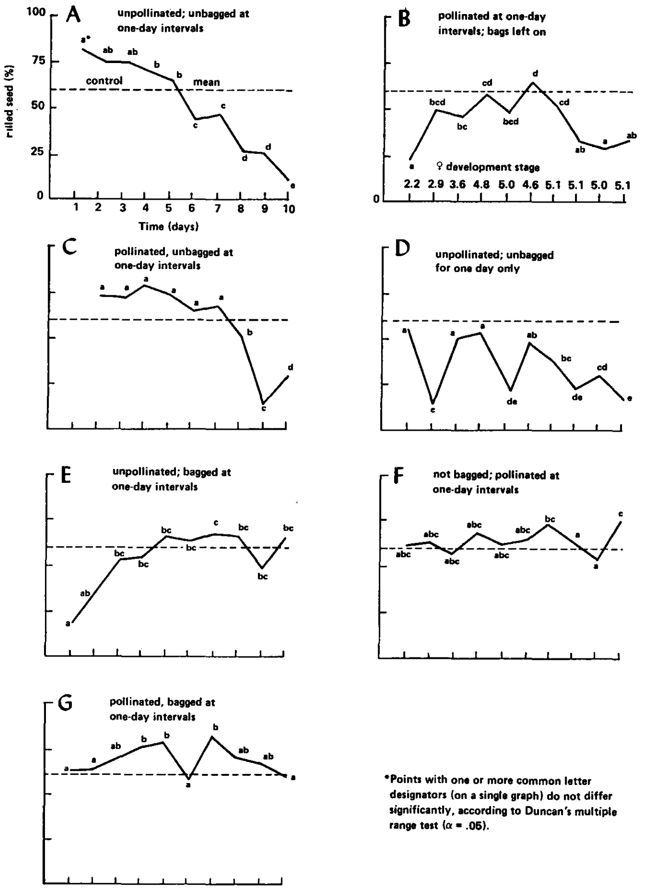
Regime	D	B	A	E	C	X	F	G
Mean:	37.0	43,3	49.6	55.2	56.6	59.5	64.5	68.3

Only two regimes (B and D) differ significantly from the control (X); both have significantly lower FS% values. This is not surprising since both B and D represent very restricted pollination regimes (see description of regimes under "Materials and Methods") and the control was open-pollinated. More important is the fact that mean FS% of regime F, which best represents supplemental mass-pollination, is not significantly greater than that of the control. However, before concluding that supplemental mass-pollination is a waste of time, regimes E, F, and G should be compared.

Regimes E and G are similar in that female strobili were bagged progressively over the ten-day treatment period. They differ only in that G treatments were supplementally pollinated at time of bagging, and E treatments were not. F treatments were not bagged at all, but were supplementally pollinated. In terms of mean FS%, G significantly exceeds E but not F; and F exceeds E but not G. This seems to indicate that when wind pollination is unimpeded, supplemental mass-pollination has little if any effect on filled seed production. But if wind pollination is restricted for any reason (e.g., poor pollen production or non-synchronous male and female flowering), filled seed production might be significantly increased by means of supplemental pollination.

Comparison of Treatments within Regimes

Results of range tests of treatment means within individual pollination regimes are shown in *Table 2* (in "test" column). In *Figure 2*, treatment means are graphed for each of the seven pollination regimes to better illustrate treatment effects and to facilitate comparison of regimes. In the following descriptions, treatments are denoted alpha-numerically (viz. regime — day, e.g., B2). "Treatment" and "day" are used synonymously.



*Points with one or more common letter designators (on a single graph) do not differ significantly, according to Duncan's multiple range test (α = .05).

Fig. 2. — Graphs depicting mean filled seed percent by treatment (day) for seven pollination treatment regimes (A through G).

Regime A

All female strobili were bagged at the outset. Ten percent were unbagged each day of the ten-day period; no artificial pollination was applied. *Figure 2A* clearly depicts a steady decline in FS% over the ten-day period, from 81% (well above the level of the open-pollinated control on Day 1 to 8% on Day 10. There is in fact a very strong negative correlation (r = —0.815***) between FS% and treatments (i.e., the number of days strobili remained in bags). A linear regression line was fitted to the data by the "least squares" method with Y-intercept a = 94.24 and slope b = —7.92 *(Figure 3A)*. This indicates the marked cumulative effect of wind pollination, with each additional day of exposure to wind pollination causing an increase in FS% of eight percentage points.

Regime B

All female strobili were bagged at the outset and throughout the ten-day period. Ten percent of the bagged strobili were artificially pollinated each day, each bag receiving only a single pollen application. *Figure 2B* illustrates the effects of varying date of pollination on FS%. There is no significant linear correlation between FS% and date of pollination *(Figure 3B)*. However, there is an apparent curvilinear relationship which reflects the temporal pattern of female strobilus receptivity. Developmental stages of female strobili (see descriptions under "Materials and Methods") are noted on *Figure 2B*. Obviously, this numerical scale of development, based only a casual observation of changes in gross morphology, has serious shortcomings as a measure of female receptivity.

Treatments B4 through B10 have essentially the same female development stages (~5); but B8, B9, and B10 have significantly lower FS% values than B4, B6, and B7. This indicates that the sharp decline in receptivity that occurred

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[2]) Any two means not underscored by the same line are significantly different (α = .05); any two means underscored by the same line are not significantly different.

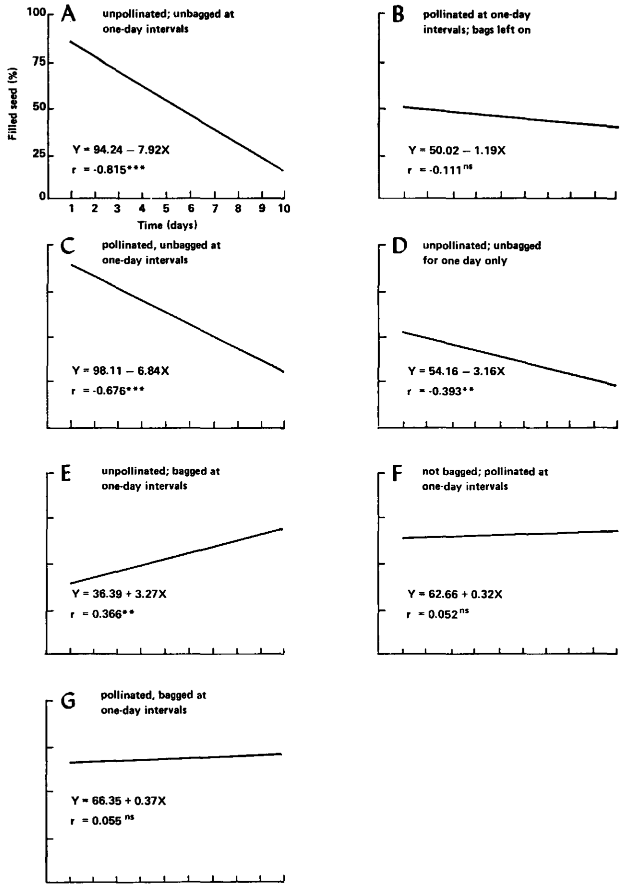

Fig. 3. — Graphs depicting linear regression of filled seed percent on time (days) for seven pollination treatment regimes (A through G).

between Days 7 and 8, as evidenced by a significant decrease in FS%, was not signalled by any obvious change in gross appearance of female strobili.

The numerical scale is somewhat more useful as an indicator of the onset of female receptivity. The significant increase in FS% between Days 1 and 2 is associated with a perceptible change in female development stage (2.2 to 2.9). There is the possibility that onset of receptivity is less abrupt and even more visually perceptible than is indicated by these data, due the likelihood of "carryover pollination".

Pollen injected into a bag on Day 2, for example, when strobili may have been relatively unreceptive, might have survived to effect pollination a day or two later when strobili were more receptive. This carryover pollination effect may also explain the high FS% values achieved with single pollen application in treatments B2 through B7, which approximate the mean of the open-pollinated control.

Regime C

All female strobili were bagged at the outset. Ten percent were artificially pollinated each day of the ten-day period, and the bags were removed immediately after pollination. This regime differs from Regime A in that C treatments included supplemental pollination and A treatments did not. *Figure 2C* shows a gradual decline in FS% over the nine-day period, form 72.6% on Day 2 (C1 data were excluded because of insect effects) to 29.6 on Day 10. As in Regime A, there is a highly significant negative correlation (r = —0.676***) between FS% and the number of days that strobili were bagged. The linear regression line *(Figure 3C)* has a greater Y — intercept (*a* = 98.11) and a lower slope (*b* = —6.84) than the corresponding A-line *(Figure 3A)*, however only the *a* values differ significantly (*a* = .05). The greater elevation (i.e., Y — intercept) of the C-line indicates that supplemental pollination did significantly increase FS% of C treatments over that of wind-pollinated A treatments. Furthermore, it is apparent from comparison of r^2 values that variation in number of days of open pollination accounts for less than half (46%) of the variation in FS% of C treatments; but the linear regression explains two-thirds of the variation in A treatments. This, too, indicates a positive effect of supplemental pollination.

This supplemental pollination effect is evident when the intersections of treatment (solid) and control (broken) lines are compared in *Figures 2A* and *2C*. In C, FS% does not drop below control until Day 8; whereas in A, this drop-off occurs two days earlier, apparently as a result of inadequate wind pollination.

Regime D

All female strobili were bagged at the outset. Ten percent were unbagged one day and rebagged the following day. No artificial pollination was applied. This treatment was intended to measure the effect of a single day's wind pollination on FS%. Figure 2D illustrates the abrupt diurnal fluctuations in FS%, varying from 55.8% on Day 1 to 14.7% on Day 2 to 50.7% on Day 3. The very high D1 value is inconsistent with the much lower values for B1 (24.5%) and E1 (18.0%) which indicate that female receptivity, not pollen supply, is the limiting factor at this early date. However, the large D1 value cannot be attributed to more advanced female receptivity, since all three treatments had very similar female development stages. Neither can the sharp drop in FS% between D1 and D2 be attributed to differences in female receptivity. The very low FS% value for D2 (14.7%) is probably the result of the suppressant effect of heavy rainfall (0.82") on natural pollen dispersal.

A highly significant negative correlation (r = —0.393**) exists between FS% and date of open pollination *(Figure 3D)*; but only 15% of the variation in FS% is attributable to date of open pollination. The obviously large deviations from the regression line are probably caused by macro- and micro-environmental variation affecting daily pollen availability and female receptivity.

Regime E

All female strobili were exposed (i.e., not bagged) at the outset. Ten percent were bagged each day. No artificial pollination was applied. This regime is the inverse of Regime A; and a comparison of *Figures 2E* and *2A* shows their reciprocal effects.

In E, FS% increases over time, rising sharply from 18.0% on Day 1 to the approximate level of the open-pollinated control on Day 3. E3 represents something more than three days of open pollination since the strobili were not bagged initially, and some pollination probably occurred even before initiation of the experiment on Day 1, as evidenced by the very high D1 value (55.8%). The plot of E values appears to be more curvilinear than that of A treatments, and this is very evident in a comparison of linear correlations *(Figures 2A* and *2E)*. The significant positive correlation between FS% and E treatments is much weaker (0.366**) than the negative correlation in A (—0.815***).

Regime F

All female strobili were exposed to wind pollination at the outset and throughout the experiment. Ten percent were artificially pollinated each day.

It is clear from *Figure 2F* that FS% values for F treatments do not differ significantly among themselves or in relation to the mean of the open-pollinated control. There is no linear correlation between FS% and treatments *(Figure 3F)*. This indicates that a single application of pollen to unbagged strobili did not appreciably increase FS%, regardless of the timing of that pollen application.

Regime G

All female strobili were exposed at the outset. Ten percent were bagged each day and artificially pollinated immediately after bagging. This regime differs from Regime E in that G treatments were supplementally pollinated and E treatments were not.

Comparison of *Figures 2G* and *2E* shows the positive effects of supplemental pollination on FS%. G1 and G2 are elevated to the level of the open-pollinated control; whereas E1 and E2 fall well below the control line. G4, G5, and G8 are significantly higher than the control (the sharp dip in the curve at G6 cannot be explained). Why these three G treatments exceed the control when their counterparts in regimes E and F do not can be explained in terms of the aforementioned "carryover pollination" effect.

Pollination bags serve not only to isolate strobili from windborne pollen, but also trap and hold the pollen injected into them, thereby permitting subsequent pollination of developing strobili over a period of several days. Consequently, Regime G sheds some light on one mode of pollination that should have been included in this experiment but was not, viz. multiple pollen applications to the same plots.

Figure 3G illustrates the lack of significant linear correlation between FS% and treatments in this regime, as contrasted to the significant positive correlation that exists in Regime E *(Figure 3E)*. It appears that supplemental pollination negated this linear correlation and increased the overall mean FS% of Regime G (68.3%) significantly over that of Regime E (55.2%).

Discussion and Conclusions

From the foregoing descriptions and comparisons of eight experimental pollination regimes, some tentative conclusions may be drawn regarding the efficacy of supplemental mass-pollination in Douglas-fir seed orchards. Under certain conditions, wind pollination may be so effective — due to its cumulative effect over time — that artificial supplementation would have no appreciable effect on filled seed percent. Such conditions might prevail in older orchards during years of abundant pollen production. But even in such years, open pollination may be inhibited to some extent as a result of non-synchronous flowering among orchard clones.

In this study, a single clone was used, and it was selected because its flowering period was somewhat intermediate in the array of orchard clones. Had a different clone, with a very early or late flowering period, been studied, supplemental pollination probably would have been much more effective, relative to wind pollination alone. So, even in the best of years, orchard seed production might be increased by supplementally pollinating early and late-flowering clones in older orchards. In young orchards, where pollen production is frequently quite limited, supplemental pollination would almost certainly increase production of filled seeds.

The effectiveness of wind pollination depends on a more or less continuous supply of pollen over the entire period of female flowering in the orchard. Just as flowering time varies among clones, not all female strobili on a given tree, or even a single branch of that tree, are receptive to pollination at any one time. In fact, not all ovules within a single strobilus may be accessible to pollen on a given day. For the single clone used in this study, the period of female receptivity exceeded the ten-day duration of the experiment. In an orchard containing 50—100 clones, pollination may extend over a period of three to six weeks. Therefore, proper timing is probably the key to maximizing effects of supplemental mass-pollination.

Ideally, supplemental pollination would be geared to the flowering of the individual orchard clones, so that several pollen applications might be made during the period of peak female receptivity in each clone. However, the practicability of such an approach is questionable at the present time. Current methods are too crude for routine use in large-scale orchard operations. Special equipment and procedures are needed to facilitate proper timing and repeated applications while conserving the costly pollen supply.

As an example of what might be possible in forest-tree seed orchards, consider what has already been achieved in commercial date palm gardens in California. Dates are conventionally produced by hand pollination, but labor shortages have forced a move toward mechanization in recent years. A mechanical pollinator (which also functions as a pesticide duster) was developed and is now used commercially, at a savings of 30 to 50% of the cost of hand pollination. Two men and a machine can service 60 to 80 acres, applying pollen 12 times per season on a twice-weekly basis (Perkins and Burkner, 1974).

Supplemental mass-pollination clearly has potential as a method for increasing the quantity and genetic quality of seed produced in wind-pollinated forest-tree seed orchards. But under what conditions will it be economically feasible? In the date palm example, mechanized pollination effectively reduced costs and increased returns relative to costly hand pollination. But in wind-pollinated tree-seed orchards, supplemental pollination is inevitably an added cost, with uncertain returns depending on individual orchard conditions and specific techniques used. Consequently, each orchardist must assess the practicality of supplemental mass-pollination, carefully weighing anticipated benefits against associated costs.

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