a need to investigate possible site × genotype interactions and to determine whether the relative resistance of the several populations, families and individual trees to WGR remains stable when exposed to collections of *E. harknessii* from a variety of geographical and host origins. Application of knowledge gained in this study to disease control in North America, and the assessment of tree breeding as a management option if WGR is accidentally introduced into other *P. radiata* - growing regions, will depend on the outcome of such research.

With the foregoing limitation, the main findings and implications for management of *P. radiata* are as follows:

This study has confirmed previous indications that the two island provenances of *P. radiata* are relatively resistant to infection by *E. harknessii* at the Russell Reservation (OLD 1981). Of the three mainland provenances, the Año Nuevo population ranked as the most resistant, and the Cambria population as the most susceptible. The lack of significant replication × population and replication × family interactions suggest that these differences exist at both high and low inoculum intensities, as replication 1 was undoubtedly subjected to a substantially greater inoculum load than were replications 3 and 4 (OLD, LIBBY and RUSSELL 1985).

There was substantial within-population variation in disease incidence and, for the select New Zealand and Australian families, highly significant between-family variation coupled with a high estimate of heritability. Analyses of limited numbers of families within the main NZ-Aust breeding design and within two of the population-samples, tested for the relative presence of additive and non-additive components of genetic variation. Although the available degrees of freedom are low the data suggest that inheritance of WGR susceptibility is largely additive.

These features of the inheritance of resistance and susceptibility to WGR indicate likely benefits from selection among tree genotypes already or concurrently selected for favourable silvicultural characteristics, and their inclusion in open-pollinated seed-orchards. Clonal selection of specific outstanding trees may remain an attractive option, particularly if sufficient additional maturation-related resistance can be added to the additive genetic gains obtained by mating selected individuals (ZAGORY and LIBBY 1985). The selection-plus-clonal option for the control of WGR runs a risk of breakdown of resistance to a variable pathogen population in the evvent of broadscale deployment of uniform radiata pine genotypes. However a proper deployment of enough unrelated clones may be no more dangerous than (or even preferable to) using seed-orchard seedlings (LIBBY 1982). Neither of these strategies is likely to achieve total resistance to the disease; however an attainable goal might be the reduction of average susceptibility of most plantations such that appropriate silvicultural practices could limit WGR-related damage to acceptable levels.

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The Effect of Crown Segment on the Mating System in a Douglas-Fir (Pseudotsuga menziesii (Mirb.) Franco) Seed Orchard

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Abstract

The mating system in a mixed clonal/seedling Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] seed orchard was studied with the aid of allozyme polymorphisms at six loci (EST-1, PGI-2, G6PD, 6PGD-1, PGM, and IDH). Trees were subjected to cooling treatment to retard bud development and compact the pollination period. The outcrossing rate of two crown levels (upper and lower) and aspects (northern and southern) were estimated and compared for clonal and seedling material separately using single and multilocus methods. Single locus estimates of $\hat{t}$ ranged

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from 0.645 to 0.999 and were significantly ($P < 0.05$) heterogeneous. No significant differences among the multilocus estimates ($\hat{t}_m$) were observed between crown levels or aspects for either the clonal or seedling trees. For all comparisons, the single locus, unweighted or weighted means ($\bar{t}$) were lower than those obtained by the multilocus method, indicating the presence of other types of consanguineous matings in addition to selfing. In general, higher outcrossing rates were observed in the clonal (0.939—0.961) than the seedling trees (0.901—0.932). The implications of these results for seed orchard management are discussed.

Key words: Pseudotsuga menziesii, seed orchard, allozymes, outcrossing rates, heterozygosity.

Zusammenfassung

Es wurde das Kreuzungssystem in einer gemischten Klon-Sämlings-Douglasien-Samenplantage *(Pseudotsuga menziesii* (MIRB.) FRANCO) mit Hilfe der Allozym-Polymorphismen an 6 Loci (EST-1, PGI-2, G6PD, 6PGD-1, PGM, IDH) untersucht. Die Bäume wurden einer Kältebehandlung unterworfen um die Knospenentwicklung zu verzögern und die Bestäubung auf einen kurzen Zeitraum zu verdichten. Die Fremdungsrate wurde in zwei Kronenstufen und in Nord-Süd-Richtung geschätzt und für das Klon- und Sämlingsmaterial verglichen, indem man die Einzel- und Multilocusmethode getrennt anwandte. Die Einzellocus-Schätzwerte von $\hat{t}$ reichten von 0.645 bis 0,999 und waren signifikant heterogen ($P < 0,05$). Für die Kronenstufen und die Richtung wurden keine signifikanten Unterschiede zwischen den Multilocus-Schätzwerten ($\hat{t}_m$) sowohl für die Sämlinge als auch für die Klone beobachtet. Für alle Vergleiche waren der Einzellocus, das ungewichtete oder gewichtete Mittel ($\bar{t}$) niedriger, als die bei der Multilocus-Methode erhaltenen Werte, was anzeigt, daß das Vorhandensein anderer Typen eng verwandter Kreuzungen zusätzlich zur Selbstung gegeben ist. Generell wurden bei den Klonen mit 0,939—0,961 höhere Fremdungsraten beobachtet als bei den Sämlingen (0,901—0,932). Die Anwendungen dieser Ergebnisse auf das Samenplantagen-Management werden diskutiert.

Introduction

Reproductive bud phenology and relative position of male and female strobili have considerable effect on the quality and quantity of seed produced in coniferous seed orchards (FOWLER 1965a, b; SHEN *et al.* 1981; EL-KASSABY *et al.* 1984). In several seed orchards, long pollination periods and lack of synchronization in the development of male and female strobili have resulted in panmictic disequilibrium (ERIKSSON *et al.* 1973; JONSSON *et al.* 1976, SHEN *et al.* 1981; O'REILLY *et al.* 1983; EL-KASSABY *et al.* 1984). The pollination period in Douglas-fir *[Pseudotsuga menziesii* (MIRB.) FRANCO] has been reported to extend over 38 days in a natural stand (GRIFFITH 1968) and 29 days in a seed orchard (EL-KASSABY *et al.* 1984). WHEELER (1984) stated that female cone receptivity began earlier in the upper than in the lower crown and, to a lesser extent, began earlier on northern than on southern aspects in one Douglas-fir seed orchard. Selfing has been reported to be higher in the lower part of the crown than the upper part due to the relative position of reproductive strobili in the crown (FOWLER 1965; FRANKLIN 1971; HADDERS 1971; SHEN *et al.* 1981; SQUILLACE and GODDARD 1982; SHAW and ALLARD 1982a, OMI 1983).

The utilization of an overhead irrigation mist (cooling) system to delay bud development within a Douglas-fir seed orchard (SILEN and KEANE 1969) has proved to be beneficial in minimizing contamination from local pollen (FASHLER and DEVITT 1980; EL-KASSABY and RITLAND 1986), in shorting the pollination period within the orchard and in reducing

the phenological variation within crown and among trees (FASHLER and EL-KASSABY, in preparation). The object of this paper is to compare the genetic composition and rates of outcrossing for viable seed samples collected from two crown levels and aspects after utilization of the cooling treatments.

Materials and Methods

Orchard Description and Cone Collections

Material for this study was obtained from the 3.4 ha. high-elevation Douglas-fir seed orchard of CIP Forest Products Inc. (formerly known as Pacific Forest Products Ltd.), Saanichton, B. C. (latitude 48⁰ 35' N, longitude 123⁰ 24' W, elevation approx. 50 m). The orchard breeding population consists of combined clonal/seedling material representing 80 plus-trees selected between elevations range of 450—1000 m on southern Vancouver Island and the south coastal mainland of B.C. Trees were planted in a randomized incomplete block design replicated 16 times (blocks). Blocks along the southern edge of the orchard consist almost entirely of clonal material, while the northern part is planted with seedlings following wind-pollination of the ortets *in situ* ("seedlings"). The remaining blocks contain a mixture of seedlings and grafted trees of differing proportions. The average ages of the clonal propagules and the seedlings are 17 and 14 years, respectively. Maximum tree height is approximately 8 m.

A special feature of this orchard's management is the availability of a solid-set overhead irrigation system, which was used in 1983 to provide a cooling treatment during reproductive bud development. The irrigation system was used for cooling between January and April, 1983 to both delay the reproductive bud phenology and to provide for frost protection. It was observed also that the reproductive phenology variation within crown was reduced and among trees was shortened to 19 days vs. 29 days in years where the system was not operational (FASHLER and EL-KASSABY, in preparation).

In September 1983, during a "bumper" cone crop, cone samples were collected from every cone-bearing tree within six randomly selected blocks. A total of 226 seedlings and 76 ramets were sampled, representing 64 out of the 80 plus trees in the orchard population. Each tree's crown was stratified into four sections prior to cone collection: north and south aspects and upper and lower crown.

Electrophoretic Methods

Electrophoretic procedures, staining recipes, and enzyme nomenclature used were those reported by EL-KASSABY *et al.* (1982a). The enzyme systems studied were: esterase (EST) E.C.3.1.1.1.; phosphoglucoisomerase (PGI) E.C.5.3.1.9; glucose-6-phosphate dehydrogenase (G6PD) E.C.1.1.1.49; 6-phosphogluconic dehydrogenase (6PGD) E.C.1.1.1.44; phosphoglucomutase (PGM) E.C.2.7.5.1; and isocitrate dehydrogenase (IDH) E.C.1.1.1.42. A total of six loci (EST-1, PGI-2, G6PD, 6PGD-1, PGM, and IDH) were censused for both megagametophytic and corresponding embryonic tissues of each viable seed. The mode of inheritance and lack of linkage of these loci are reported by EL-KASSABY *et al.* (1982a, b). Where possible, a total of eight seeds were assayed for every crown quadrant· yielding a total of 16 seeds per crown segment (upper vs. lower or northern vs. southern) per tree.

Estimation of Mating System

The multilocus, mixed-mating model for estimating outcrossing rate and outcrossing pollen gene frequencies de-

veloped by RITLAND and EL-KASSABY (1985) was used to estimate the mating-system parameters for the different crown segments in clonal and seedling trees. The model is a multilocus extension of the single locus "conifer" models of EL-KASSABY *et al.* (1981), SHAW and ALLARD (1982a), and CHELIAK *et al.* (1983) that include the gametophytic contribution to the embryo's genetic structure. For m cencused zygotes, the multilocus likelihood equation for this model is of the form $\prod\limits_{i=1}^{m} (tT_i + (1 - t) S_i)$ where t is the outcrossing rate, T_i is the probability of the multilocus zygote i given it was outcrossed, and S_i is the probability of the multilocus zygote i given it was selfed (both probabilities are also conditioned upon known maternal parentage). For n loci, T_i consists of the product of single-locus probabilities of observing zygote i at locus j given that the zygote was outcrossed, for $j = 1$ to n, and S_i consists of the product of single-locus probabilities of observing zygote i at locus j given that the zygote was selfed, for $j = 1$ to n. These probabilities are determined by Mendelian rules of inheritance with the added consideration of megagametophyte contributions. Explicit formulae and statistical properties of the estimates are given in RITLAND and EL-KASSABY (1985). Single locus outcrossing rates were calculated using the same model applied to each locus separately. Allelic frequencies of outcrossing pollen, maternal, and embryonic gene pools were compared using the chi-square goodness-of-fit test (STEEL and TORRIE 1980).

Results and Discussion

For the six loci studied, allelic frequencies of observed outcrossing pollen, maternal (estimated from maternal trees' genotypes), and progeny gene pools were calculated for the upper and lower crown levels *(Table 1)* and northern and southern crown aspects *(Table 2)* of clonal and seedling trees. The maternal allelic frequencies differed slightly between "upper and lower crown" *(Table 1)* and between "northern and southern aspects" *(Table 2)* due to the inclusion of some different trees in each category. No

significant differences were observed in the maternal gene pools between either the two crown levels or the two aspects at any of the loci. In the pollen pools, significant differences (P < 0.05) were observed for some loci between upper and lower crown levels [EST-1 (clonal and seedling)] and between northern and southern aspects [G6PD (clonal) and EST-1 and IDH (seedling)]. For the progeny gene pools, significant differences (P < 0,05) were observed between crown levels [EST-1 (seedling)] and between aspects [EST-1 and IDH (seedling)]. Since the allelic frequencies of the maternal gene pools did not differ and the lack of independence of the progeny allelic frequency is due to association with maternal genotypes, the observed differences in the progeny gene pools may be attributed to differences in the pollen pools. In fact, all of the three significantly different loci in the progeny pool were also significantly different in the pollen pool.

When the progeny and maternal allelic frequencies were compared, no significant differences were observed for any crown segment in clonal or seedling trees. Differences in allelic frequency between progeny and maternal trees have been observed in a natural Douglas-fir stand (EL-KASSABY *et al.* 1981), in *Pinus radiata* D. DON (MORAN *et al.* 1980), and in *Eucalyptus* spp. (MORAN and BROWN 1980; YEH *et al.* 1983).

Allelic frequencies of the pollen and maternal gene pools were compared to determine whether the effective pollen pools were random samples of the adult trees, represented by the maternal trees. No significant differences between the two gene pools were observed between crown levels for either clonal or seedling trees. On the other hand, significant differences (P < 0.05) were observed at EST-1 for both aspects in the seedling trees. MÜLLER-STARCK *et al.* (1983) and YEH *et al.* (1983) reported several factors that could be responsible for the observed discrepancy in allelic frequencies among different gene pools. These factors include differences in pollen production or phenological development among pollen parents, differences in outcrossing pollen pools and/or the presence of gametic or zygotic (postmating) selection.

Table 1. — Allelic frequencies for outcrossing pollen, maternal, and progeny gene pools for samples obtained from the upper (U) and lower (L) portions of the crown for clonal and seedling trees.†

Locus	Allele	CLONAL TREES						SEEDLING TREES					
		U			L			U			L		
		Pollen	Maternal	Progeny	Pollen	Maternal	Progeny	Pollen	Maternal	Progeny	Pollen	Maternal	Progeny
EST-1	1	0.511	0.532	0.509	0.552	0.570	0.555	0.491	0.544	0.522	0.477	0.541	0.510
	2	0.215	0.290	0.259	0.203	0.281	0.241	0.230	0.223	0.228	0.257	0.229	0.244
	3††	0.274	0.178	0.232	0.245	0.149	0.204	0.279	0.233	0.250	0.266	0.230	0.246
PGI-2	1	0.922	0.887	0.903	0.923	0.886	0.900	0.931	0.940	0.938	0.930	0.941	0.938
	2	0.078	0.113	0.097	0.077	0.114	0.100	0.068	0.058	0.061	0.069	0.057	0.052
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.002	0.001	0.001	0.002	0.010
G6PD	1	0.499	0.524	0.516	0.513	0.535	0.541	0.476	0.460	0.463	0.472	0.466	0.470
	2	0.438	0.395	0.415	0.444	0.386	0.399	0.487	0.511	0.502	0.493	0.505	0.498
	3††	0.063	0.081	0.069	0.043	0.079	0.060	0.037	0.029	0.035	0.035	0.029	0.032
6PGD-1	1	0.904	0.903	0.902	0.889	0.912	0.891	0.903	0.894	0.900	0.903	0.894	0.899
	2	0.051	0.056	0.056	0.046	0.053	0.053	0.051	0.046	0.048	0.047	0.045	0.045
	3††	0.045	0.041	0.042	0.065	0.035	0.056	0.046	0.060	0.052	0.050	0.061	0.056
PGM	1	0.886	0.887	0.889	0.878	0.904	0.887	0.904	0.889	0.898	0.903	0.889	0.893
	2	0.077	0.105	0.088	0.082	0.096	0.094	0.042	0.053	0.048	0.053	0.054	0.054
	3	0.037	0.008	0.023	0.040	0.000	0.019	0.054	0.058	0.054	0.044	0.057	0.053
IDH	1	0.847	0.790	0.815	0.854	0.781	0.815	0.831	0.851	0.846	0.841	0.855	0.853
	2	0.077	0.137	0.110	0.082	0.132	0.108	0.092	0.069	0.077	0.091	0.068	0.076
	3††	0.076	0.073	0.075	0.064	0.087	0.077	0.077	0.080	0.077	0.068	0.077	0.071

† See Table 4 for sample size.
†† Synthetic allele (all alleles but the two most common were bulked in one class).

Table 2. — Allelic frequencies for outcrossing pollen, maternal, and progeny gene pools for samples obtained from the northern (N) and southern (S) aspects of the crown for clonal and seedling trees.†

Locus	Allele	CLONAL TREES						SEEDLING TREES					
		N			S			N			S		
		Pollen	Maternal	Progeny	Pollen	Maternal	Progeny	Pollen	Maternal	Progeny	Pollen	Maternal	Progeny
EST-1	1	0.546	0.554	0.543	0.514	0.553	0.520	0.501	0.544	0.521	0.467	0.544	0.512
	2	0.204	0.269	0.249	0.231	0.273	0.260	0.217	0.223	0.224	0.270	0.223	0.247
	3††	0.250	0.177	0.208	0.255	0.174	0.220	0.282	0.233	0.255	0.263	0.233	0.241
PGI-2	1	0.915	0.900	0.905	0.929	0.886	0.903	0.933	0.940	0.940	0.927	0.940	0.928
	2	0.084	0.100	0.094	0.071	0.114	0.097	0.066	0.058	0.059	0.072	0.058	0.062
	3	0.001	0.000	0.001	0.000	0.000	0.000	0.001	0.002	0.001	0.001	0.002	0.010
G6PD	1	0.472	0.523	0.509	0.544	0.530	0.540	0.469	0.460	0.466	0.479	0.460	0.468
	2	0.468	0.400	0.426	0.416	0.402	0.403	0.495	0.511	0.501	0.485	0.511	0.498
	3††	0.060	0.077	0.065	0.040	0.068	0.057	0.036	0.029	0.033	0.036	0.029	0.034
6PGD-1	1	0.892	0.923	0.899	0.903	0.909	0.895	0.904	0.894	0.898	0.902	0.894	0.901
	2	0.050	0.046	0.054	0.049	0.061	0.060	0.050	0.046	0.047	0.048	0.046	0.045
	3††	0.058	0.031	0.047	0.048	0.030	0.045	0.046	0.060	0.055	0.050	0.060	0.054
PGM	1	0.876	0.892	0.886	0.885	0.902	0.890	0.905	0.889	0.898	0.903	0.889	0.894
	2	0.086	0.100	0.093	0.074	0.090	0.088	0.049	0.053	0.051	0.046	0.053	0.051
	3	0.038	0.008	0.021	0.041	0.008	0.022	0.046	0.058	0.051	0.051	0.058	0.055
IDH	1	0.852	0.800	0.824	0.843	0.796	0.809	0.847	0.854	0.858	0.825	0.854	0.841
	2	0.070	0.123	0.098	0.090	0.136	0.116	0.085	0.069	0.071	0.097	0.069	0.081
	3††	0.078	0.077	0.078	0.067	0.068	0.075	0.068	0.077	0.071	0.078	0.077	0.078

† See Table 4 for sample size.
†† Synthetic allele (all alleles but the two most common were bulked in one class).

The relationship between observed (H) and expected (h) frequencies of heterozygotes in the progeny was used to measure the extent of inbreeding in the progeny. WRIGHT's (1922) fixation index F was calculated for the viable progeny collected from each crown level and aspect for both the clonal and seedling trees using the formula $F = 1 - (H/h)$, where H is the observed frequency of heterozygotes and h is the expected frequency of heterozygotes under panmixis (Table 3). The variance of F was calculated from the inverted information matrix (KENDALL and STUART 1979) and the 95% confidence interval was estimated for each F value for each locus.

In general, the fixation index under panmixia is equal to zero, and will be increased by inbreeding and decreased by selection favoring hetrozygotes. Statistically significant, positive, F values arise from either inbreeding due to self-fertilization or differences in allelic frequencies in the effective pollen pool of each tree (the WAHLUND effect (LI, 1955)).

In the clonal trees, significant (P < 0.05) positive F values were obtained for EST-1 in the upper crown and EST-1 and PGI-2 in the lower crown (Table 3). The mean of F values were 0.047 and 0.056 for upper and lower crown, respectively, indicating 4.7 and 5.6% deficiencies of heterozygotes relative to Hardy-Weinberg expectation. Significant (P < 0.05) positive F values occurred at EST-1 in both aspects (Table 3). The mean F values were 0.045 and 0.048 for northern and southern aspects, respectively.

In the seedling trees, significant (P < 0.05) positive F values were observed at EST-1 and 6PGD-1 for the upper and EST-1, PGI-2, 6PGD-1, and PGM for the lower crown; significant positive F values were found also for EST-1, PGI-2, and PGM for northern and EST-1, PGI-2, and 6PGD-1 for the southern aspects. The mean F values were 0.063 and 0.058 for upper and lower crowns. respectively, and 0.059 for both aspects. Whether data are analyzed by crown level or by aspect (Table 3), more statistically-significant, positive, F values are observed for seedling trees than for clonal trees. This pattern indicates that inbreeding may be slightly higher in the seed crop from the seedling trees.

EST-1 was the only locus that show significant (P < 0.05) departure from Hardy-Weinberg expectations in each crown level and aspect for both clonal and seedling trees (Table 3). Such a consistent pattern of excessive homozygosity was also found at this locus in other studies on Douglas-fir (SHAW and ALLARD 1982b; F. C. YEH, pers. comm.) and on loblolly pine (Pinus taeda L.) (ROBERDS and CONKLE 1984). The cause of this pattern is unknown for Douglas-fir. However, in loblolly pine, ROBERDS and CONKLE (1984) suggested that natural selection may have been the contributing factor by acting either directly on the EST locus or at correlated loci. The esterase protein and its developmental function were reported to exhibit abnormal genetic behaviour (TEMPLETON, cited in MARX 1984) as well as unusual segregation in Pinus virginiana MILLER (WITTER and FERET 1979). Further examination of our data for this locus revealed that megagametophytes were being produced at the expected 1:1 ratio. RITLAND and EL-KASSABY (1985) have concluded that the EST-1 locus is not a neutral genetic marker appropriate for measuring inbreeding.

Single locus ($\hat{t}$) and multilocus ($\hat{t}_m$) estimates of outcrossing rates are presented in Table 4. Except for the EST-1 locus, single locus estimates of outcrossing rates were higher than 0.898; results similar to those obtained by EL-KASSABY et al. (1981) and SHAW and ALLARD (1982a) for the same species. The single locus estimates did not differ significantly among loci when estimates based on the EST-1 locus were excluded. Significant differences among loci have been reported for Douglas-fir (EL-KASSABY et al. 1981; SHAW and ALLARD 1982a); Pinus radiata (MORAN et al. 1980); Pinus ponderosa Laws. (MITTON et al. 1981); Pinus contorta DOUGL. (EPPERSON and ALLARD 1984); and several Eucalyptus spp. (BROWN et al. 1975; PHILLIPS and BROWN 1977; MORAN and BROWN 1980; YEH et al. 1983). This observed variation is an inherent problem of all single locus estimates due to their sensitivity to any violation to the mixed mating model's assumption (FYFE and BAILEY 1951; BROWN and ALLARD 1970;

| Locus | CROWN LEVEL | | | | CROWN ASPECT | | | |
| | Clonal Trees | | Seedling Trees | | Clonal Tree | | Seedling Trees | |
	U	L	U	L	N	S	N	S
EST-1	0.258 ± 0.054*	0.221 ± 0.059*	0.243 ± 0.027*	0.199 ± 0.028*	0.275 ± 0.056*	0.195 ± 0.057*	0.238 ± 0.028*	0.205 ± 0.028*
PGI-2	0.017 ± 0.068	0.179 ± 0.098*	0.069 ± 0.045	0.060 ± 0.043*	0.029 ± 0.073	0.069 ± 0.081	0.053 ± 0.043*	0.068 ± 0.044*
G6PD	-0.032 ± 0.058	-0.018 ± 0.062	0.004 ± 0.029	-0.011 ± 0.030	-0.040 ± 0.060	-0.006 ± 0.059	0.001 ± 0.029	-0.006 ± 0.029
6PGD-1	0.028 ± 0.060	-0.010 ± 0.057	0.038 ± 0.031*	0.043 ± 0.032*	0.011 ± 0.055	0.010 ± 0.055	0.027 ± 0.030	0.044 ± 0.032*
PGM	-0.010 ± 0.055	0.025 ± 0.064	0.027 ± 0.029	0.036 ± 0.031*	0.001 ± 0.054	0.010 ± 0.057	0.032 ± 0.030*	0.026 ± 0.029
IDH	0.022 ± 0.055	-0.009 ± 0.058	-0.004 ± 0.026	0.019 ± 0.028	-0.007 ± 0.054	0.012 ± 0.056	0.001 ± 0.026	0.014 ± 0.027
Mean	0.047	0.056	0.063	0.058	0.045	0.048	0.059	0.059

* Rejection of the null hypothesis that $F = 0.00$ at 5% level.

EPPERSON and ALLARD 1984). Unweighted means of single locus estimates $(\bar{t})$ varied between 0.884 and 0.924 depending on crown segment and type of parent trees (Table 4). The overall unweighted means are very close to mean single locus estimates of 0.9 previously reported by EL-KASSABY et al. (1981), SHAW and ALLARD (1982a), and OMI (1984) in several natural stands and two clonal seed orchards.

The multilocus estimates of outcrossing rates for all crown segments in clonal and seedling trees varied between 0.901 and 0.961 with an overall unweighted mean of 0.934 (Table 4). No significant differences were observed between the $\hat{t}_m$ estimates for upper and lower crown levels or northern and southern aspects in either the clonal or seedling trees (Table 4)). On average, $\hat{t}_m$ was slightly higher, though not significant, in the upper crown than in lower crown in both clonal and seedling trees (0.5—3%) (Table 4). These differences were similar to or less than those re-ported previously on "uncooled" Douglas-fir clonal orchards (3%, non-significant by SHAW and ALLARD 1982a; 13%, significant by OMI 1983). Since our orchard was under cooling treatment, a reduction in within-tree reproductive phenological differences as well as shortening of the pollination period could be responsible for the lack of differences in the outcrossing rate between upper and lower crown reported here. OMI (1983) recommended that cone collection from the lower crown should be ignored based on the observed high proportions of selfed seeds in the lower crown which was accompanied by a low estimate of filled seed.

Estimates of $\hat{t}_m$ for individual trees varied between 0.292 and 1.300 (data based on a mean of 29.3 seeds per tree) (Figure 1). This wide variation in $\hat{t}_m$ estimates among individual trees was reported also by SHAW and ALLARD (1982a) and OMI (1983). SHAW and ALLARD (1982a) related these differences to the high individual variability in self-fertility ob-

| Locus | CROWN LEVEL | | | | CROWN ASPECT | | | |
| | Clonal Trees | | Seedling Trees | | Clonal Tree | | Seedling Trees | |
	U	L	U	L	N	S	N	S
EST-1	0.712 (0.030)	0.615 (0.028)	0.721 (0.014)	0.665 (0.013)	0.645 (0.027)	0.731 (0.028)	0.680 (0.014)	0.707 (0.014)
PGI-2	0.930 (0.027)	0.976 (0.017)	0.900 (0.005)	0.899 (0.005)	0.976 (0.023)	0.924 (0.024)	0.899 (0.005)	0.900 (0.007)
G6PD	0.983 (0.010)	0.999 (0.008)	0.981 (0.002)	0.923 (0.003)	0.999 (0.006)	0.934 (0.011)	0.934 (0.003)	0.933 (0.002)
6PGD-1	0.980 (0.010)	0.932 (0.006)	0.931 (0.005)	0.931 (0.003)	0.929 (0.005)	0.934 (0.009)	0.917 (0.005)	0.933 (0.005)
PGM	0.931 (0.027)	0.991 (0.053)	0.898 (0.004)	0.900 (0.005)	0.998 (0.047)	0.929 (0.023)	0.898 (0.005)	0.900 (0.005)
IDH	0.959 (0.010)	0.958 (0.010)	0.977 (0.005)	0.983 (0.007)	0.999 (0.013)	0.985 (0.009)	0.999 (0.006)	0.934 (0.006)
$\bar{t}$	0.916	0.918	0.901	0.884	0.924	0.906	0.888	0.885
$\bar{t}_{min}$††	0.945 (0.018)	0.942 (0.016)	0.938 (0.007)	0.896 (0.007)	0.948 (0.015)	0.928 (0.017)	0.910 (0.008)	0.908 (0.007)
$\hat{t}_m$	0.955 (0.030)	0.950 (0.021)	0.932 (0.012)	0.901 (0.012)	0.961 (0.024)	0.939 (0.025)	0.916 (0.012)	0.916 (0.011)
# of trees	57	62	221	226	65	66	226	226
# of seeds	787	911	3470	3571	871	865	3526	3526

† Standard errors in parentheses.

†† Minimum variance mean $(\bar{t}_{min}) = [\sum_{i=1}^{n} \frac{1}{V_{\hat{t}_i}}]^{-1} [\sum_{i=1}^{n} \frac{\hat{t}_i}{V_{\hat{t}_i}}]$ where n = # of loci; $\hat{t}_i$ = single locus estimate; $V_{\hat{t}_i}$ = variance of $\hat{t}_i$.
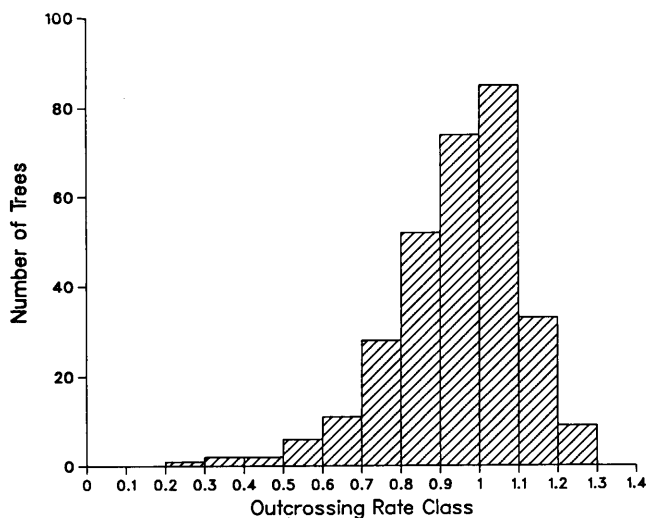
Fig. 1. — Frequency distribution of estimated outcrossing rates for 302 trees studied.

served among trees and the presence of high levels of self pollen in their orchard. Omi (1983) proposed that these "high selfers" should be rogued from the orchard or ignored during cone collection. Estimates of t greater than one are likely caused by negative assortative mating or by sampling error. Although these estimates are "biologically unreasonable", they were necessary to provide an unbiased estimate of the average t over rees. Restricting t to be equal to or less than 1.0 (Cheliak et al. 1983) is not statistically valid because this restriction will underestimate both the mean and variance of t among trees (Ritland and El-Kassaby 1985) and not realize the presence of assortative mating (Hamrick 1982).

An estimate of inbreeding in addition to selfing can be inferred by comparing estimates from the multilocus model with those from a single locus model, since the single locus estimates of $\hat{t}$ should be biased downward by any form of inbreeding in addition to selfing (Shaw et al. 1981). Such matings are expected in this study because the orchard population was derived from three sources: grafted clones, representing the total genetic makeup of 43 selected plus-trees, seedlings derived from wind-pollinated seeds collected from another set of 19 plus-trees, and clonal/seedling material representing a third group of 18 plus-trees. Therefore, other types of consanguineous matings may occur in addition to selfing, specifically between clonal and seedling trees derived from the same ortet and/or among individuals belonging to the same seedling family (see Ritland and El-Kassaby (1985) for a detailed discussion). In all crown segments, the single locus unweighted means were lower than those obtained by the multilocus method (Table 4) confirming the presence of these types of matings. Due to the low estimates of $\hat{t}$ obtained from the EST-1 locus, the unweighted means over all loci, frequently used in the literature, are unrealistic estimates. Therefore, minimum variance estimates of $\bar{t}$ ($\bar{t}_{min}$) were calculated (Table 4) and used to reassess the comparison between single locus and multilocus estimates of outcrossing rates. Once again, with the exception of the upper crown (seedling), $\bar{t}_{min}$ values were lower than t_m, indicating the presence of mating among relatives.

The estimates of outcrossing rates for clonal trees were higher than those for seedling trees (Table 4); results were in agreement with the differences observed in Wright's fixation index (Table 3). It has been demonstrated that

population structuring and plant density have a profound effect on apparent outcrossing rates in several plant species (Ellstrand et al. 1978; Ennos and Clegg 1982; Ellstrand and Foster 1983). The orchard population substructuring (clonal vs. seedling), the number of clonal (61) vs. families (37), the number of ramets per clone and seedlings per wind- pollinated family (frequency), differences in crown size between clonal and seedling trees (vigor), and effect of grafting incompatibility, which in turn produced wider spacing in the clonal part of the seed orchard (density), could be the sources of these differences in apparent outcrossing rates. The 76 ramets studied were representative of a total of 41 out of 61 clones (orchard averages 6.1 ramets/clone) while the 226 seedlings were representative of the 37 open-pollinated families in the orchard (orchard average 17.4 trees/family). Differences between the well-developed crown for the seedling trees and crowns of the grafts which showed plagiotropism (branch habit), as well as stunted growth due to grafting incompatibility, produced uneven spacing and tree size in the orchard. These probably allowed more free air movement and mixing of pollen in the clonal portion of the orchard than in the seedling portion. These several factors could have acted individually or in concert to permit a higher frequency of consanguineous mating in the seedling trees than in the clonal trees.

Differences between $\hat{t}_m$ obtained from this study and those previously reported for other Douglas-fir orchards (Shaw and Allard 1982a; Omi 1983) could be due to the use of different outcrossing rate estimation methods, number of loci studied, sampling intensity, and orchard population structure. The observed lack of significant differences in outcrossing rates obtained from different crown segments in this study may be the product of the enhancement of panmixis in the orchard population induced by the cooling treatment, which in turn produced uniformly higher $\hat{t}_m$ estimates.

Conclusion

Three important findings to seed orchard management were revealed in this study: 1) the observed lack of significant differences in the estimates of $\hat{t}_m$ between the upper and lower crown (Table 4), 2) the presence of high selfers in the orchard population (Figure 1), and 3) the outcrossing rate in the clonal portion is higher than the seedling portion of the orchard (Table 4). In addition to the use of the waterspray cooling system as an effective method to prevent pollen contamination of the orchard, the cooling system seems to improve panmixis in the orchard, increasing the outcrossing rate, and eliminating differences in outcrossing rate between different crown segments. Under this management, cone harvesting from the lower crown will not reduce the genetic quality of the seed crop. The removal of some trees from the seedling part of the orchard, especially the high selfers, will improve free air movement and may eventually increase the outcrossing rate without any appreciable effect on seed production. In the meantime, unless further study shows other results, management should be directed to maintaining the high outcrossing rate detected in the clonal portion and to increasing the outcrossing rate and decreasing the frequency of consanguineous matingn in the seedling portion.

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Gains from First-Cycle Selection in Slash and Longleaf Pines

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Summary

In tree breeding research begun in 1953, three stages of selection--initial, family, and within-family--were performed in slash (Pinus elliottii Engelm. var. elliottii) and longleaf (P. palustris Mill.) pines. Several traits were used in the selection, but no selection index was constructed. Results from three studies, two on slash and one on longleaf pines, were assessed at progeny age 21 or 22 years. Initial selec-