Low Levels of Pollen Contamination in a Douglas-Fir Seed Orchard as Detected by Allozyme Markers

By Y. A. El-Kassaby[1]) and K. Ritland[2])

(Received 27th November 1985)

Abstract

Allozyme gene markers are used to directly estimate the proportion of pollen contamination from adjacent natural stands in a Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seed orchard located on Vancouver Island, B. C. The estimation method relies upon differences of electrophoretic allele frequencies between natural stands and the seed orchard population at single loci, from which an average estimate of contamination over loci is subsequently obtained. This method ignores the additional information provided by linkage disequilibrium between loci, which is expected to be small in our situation due to the large population size. The estimated contamination rate from adjacent natural stands was 0.2% ± 5.7% for this orchard. This estimate is less than those previously reported for this orchard. It is not known whether this difference is due to problems in methodology or to year-specific factors, including the use of a cooling treatment to delay the reproductive phenology of the orchard.

Key words: Allozyme, Seed orchard, Contamination, *Pseudotsuga menziesii*, Cooling treatment.

Zusammenfassung

Allozyme (allelische Isoenzyme) sind als Genmarker verwendet, um direkt den Anteil der Pollenkontamination von benachbarten Beständen in einer Douglasien-Samenplantage (*Pseudotsuga menziesii* (Mirb.) Franco) auf Vancouver Island, B.C., zu schätzen. Die Schätzmethode beruht auf Unterschieden der elektrophoretisch ermittelbaren Allelhäufigkeiten zwischen dem natürlichen Bestand und der Samenplantagenpopulation an einzelnen Loci, bei denen ein durchschnittlicher Schätzwert der Kontamination über die Loci danach ermittelt wurde. Diese Methode ignoriert die zusätzliche Information, die durch Kopplungsungleichgewichte zwischen Loci verfügbar gemacht werden kann, die jedoch in unserem Fall wegen der großen Populationsgröße als klein angesehen werden kann. Die Schätzwerte für die Kontaminationsrate von benachbarten, natürlichen Beständen betrug 0,2% ± 5,7% für die Samenplantage. Dieser Schätzwert ist kleiner als jene, von denen früher berichtet wurde. Es ist nicht bekannt, ob dieser Unterschied auf Probleme der Methode oder auf jahresspezifische Faktoren zurückzuführen ist, einschließlich der Anwendung einer Kältebehandlung, um die reproduktiven Vorgänge in der Samenplantage zu verzögern.

Introduction

Contamination by extraneous pollen in seed orchards should be avoided (Werner, 1975). Methods used to reduce contamination include: 1) geographical isolation by establishing orchards outside the species range (Sarvas, 1970; Hadders, 1972) or at different elevations (Strand, 1957; Silen, 1963); 2) reducing the frequency of outside pollen by the use of supplemental mass-pollination (Denison, 1973; Woessner and Franklin, 1973; Bridgwater and Trew, 1981), establishing larger orchards (Wright, 1953), and surrounding the orchards by isolation zones (Wright, 1953; Squillace, 1967); or 3) physiological isolation through phenological manipulation by the use of water-spray cooling treatment (Silen and Keane, 1969; Fashler and Devitt, 1980).

As a mean to judge the efficiency of these isolation methods, recently, allozyme markers (Friedman and Adams, 1981; Smith and Adams, 1983) and monoterpenes (Squillace and Long, 1981) have been used to estimate pollen contamination in several seed orchards through direct genetic analyses. Each of these studies used a different analysis method suited for the specific situation; i.e., Squillace and Long (1981) based their analysis on the presence of new recombinant genotypes in the orchard progeny not obtainable by mating among the nine clones in the slash pine (*Pinus elliottii* Engelm.) clonal orchard; Friedmann and Adams (1982) based their analysis upon detecting unique alleles present in the surrounding natural stands in their loblolly pine (*Pinus taeda* L.) orchard progeny; and Smith and Adams (1983) utilized a multilocus method based on the principle that the number of all possible gametic combinations within their Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] clonal orchard (limited number of clones) is less than those in the natural stand (unlimited number of trees). Generally, these methods are dependent upon the appearance of unique alleles or unique genotypes in the progeny of orchard trees to detect migration of contaminating pollen.

To assess the potential effectiveness of a water-spray cooling system in a Douglas-fir seed orchard, this paper uses a method to estimate contamination in seed orchards based upon differences between single locus outcrossing pollen (male) vs. ovule (female) gene frequency estimates. Since Douglas-fir can self-pollinate, these estimates were obtained from the multilocus mixed-mating model for conifers (Ritland and El-Kassaby, 1985). This multilocus model gives more accurate estimates of outcrossing pollen gene frequency at single loci because selfs are more accurately identified and excluded from the outcrossing pollen gene pool. The method for estimation of contamination, which is a distinct problem from that of estimation of mixed-mating model parameters, may be more generally applicable than those that rely on the presence of unique alleles because unique alleles are special cases of differences in allele frequency.

Materials and Methods

Seed Orchard Description

The CIP Inc. (formerly known as Pacific Forest Products Limited) (Victoria, B. C.) orchard consists of a combined clonal/seedling breeding population derived from 80 plus trees selected from southern Vancouver Island and south coastal British Columbia over an elevation range of 450—1000 m. The orchard is arranged in a randomized incomplete block design with 16 blocks (*Figure 1*). At the time of study in 1983, ramets and seedlings averaged 17 and 14 years old, respectively, with a maximum tree height

[1]) CIP Inc., Tahsis Pacific Region, 8067 East Saanich Rd., R. R. # 1, Saanichton, B. C., Canada, V0S 1M0 and Faculty of Forestry, University of British Columbia, Vancouver, B. C., Canada V6T 1W5.

[2]) Department of Botany, University of Toronto,Toronto, Ontario, Canada, M5S 1A1.

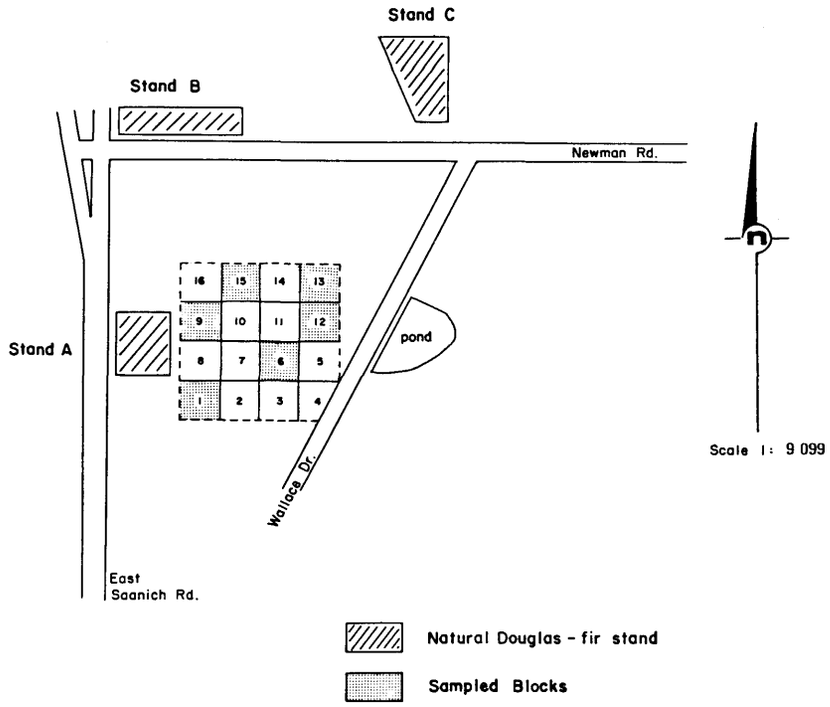

Fig. 1. — Layout of CIP Inc. seed orchard.

of 8 m. Three small Douglas-fir woodlots surrounding the seed orchard *(Figure 1)* ranged from 50 to 100 years old and from 20 to 30 m in height.

A special feature at this orchard is a solid-set overhead irrigation system used to delay the reproductive bud opening past the local flowering period so that contamination from local pollen outside the orchard is minimized (Fashler and Devitt, 1980). 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.

Cone Collections

Cone samples were collected from every cone bearing tree within six randomly selected blocks *(Figure 1)*. A total of 302 trees were sampled,representing 64 out of the 80 plus trees in the orchard population. An additional cone collection from a helicopter was made from 20, 24, and 22 trees representing the three woodlots A, B, and C, respectively *(Figure 1)*, which are putative sources of pollen contamination. The individuality of cone lots and subsequent seedlots had been retained for these trees. Cones were air dried at room temperature, seeds were extracted, dewinged and cleaned by hand and stored at 3° C until further use.

Electrophoretic Methods

Electrophoretic procedures, staining recipes, and enzyme nomenclature used followed methods reported by El-Kassaby *et al.* (1982). The enzyme systems studied were: esterase (EST) E.C.3.1.1.1; phosphogluco-isomerase (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. The mode of inheritance of these six loci have been reported elsewhere (El-Kassaby *et al.*, 1982).

The genotypes of the studied trees were determined for six loci using a sample of 8 and 32 megagametophytes per tree for the natural stands (woodlots) and the orchard trees, respectively. The probability of correctly identifying a heterozygote at a particular locus is $1-(1/2)^{K-1}$, for K megagametophytes assayed per tree. The structure of conifer seeds allowed assay of both the megagametophyte genotype (haploid) and its corresponding embryo (diploid) genotype. Calculation of both the ovule (maternal tree) and the outcrossing pollen gene gene pool frequencies for each block was performed using a multilocus mixed mating model that makes efficient use of gametophytic conifer data (Ritland and El-Kassaby, 1985). The mixed-mating model was necessary to exclude selfed pollen, which consists of about 5% of the total pollen pool (Ritland and El-Kassaby, 1985), from the migration estimate.

Contamination Model

The frequencies of the most common allele in the maternal tree population together with frequencies of the orchard (block) outcrossing pollen gene pools and the outside source (natural stand) were used to estimate the contamination rate at locus i using the following migration model *(Figure 2)*.

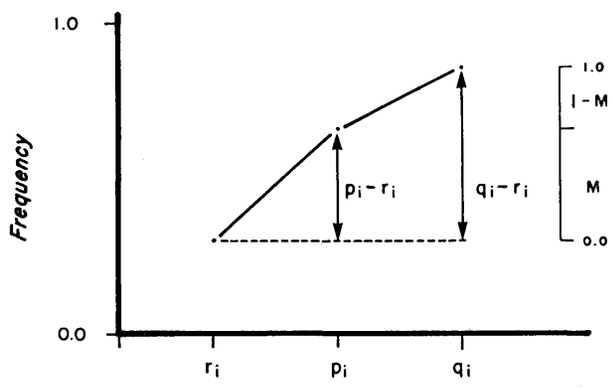

Fig. 2. — Migration model.

$$p_i = (1-m)\, r_i + m q_i \qquad (1)$$

where:

r_i = frequency of the most common allele in the orchard ovule pool (maternal tree population) at the i^{th} locus (and among pollen produced by orchard trees),

p_i = frequency of the same allele in the orchard outcrossing pollen pool at the i^{th} locus (this includes foreign pollen),

q_i = frequency of the same allele in the outside source (natural stand) at the i^{th} locus, and

m = migration rate of pollen, or proportion of natural stand pollen present in the orchard.

This model assumes that pollen produced by the orchard population has the same gene frequency as the maternal tree population in the orchard. The rate of pollen contamination (m) is estimated with the method of moments by solving the previous equation from m, giving

$$\hat{m}_i = \frac{p_i - r_i}{q_i - r_i} \qquad (2)$$

Estimates of the r_i, p_i and q_i are obtained from the progeny array data, as previously described. However, sex-differential fertility of specific allozymes violates the assumption that the pollen production by orchard trees equals r_i. If male fertility varies in the direction of the foreign pollen gene frequency the migration rate is underestimated; if it varies in the opposite way, migration is overestimated. As long as this variation is not systematic or predictable in one direction or the other, the migration estimate would be unbiased by this sex-differential fertility (particularly when $\bar{m}$ for many loci are averaged), but this variance of the migration estimate would be increased, as discussed later.

The variance of m_i was calculated using the differential approximation as follows:

$$V(\hat{m}_i) = \mathrm{Var}(p)\left(\frac{\partial \hat{m}}{\partial p}\right)^2 + \mathrm{Var}(r)\left(\frac{\partial \hat{m}}{\partial r}\right)^2 + \mathrm{Var}(q)\left(\frac{\partial \hat{m}}{\partial q}\right)^2$$

There are no covariance terms in the differential approximation of $V(\hat{m}_i)$ since p, r, and q are independent samples of genes. This variance assumed sample size is sufficiently large (>30) and gene frequencies not extreme ($0.05 \leq p_i \leq 0.95$) such that errors are normally distributed. Solving for the differential approximation gives:

$$V(\hat{m}_i) = \left[\frac{p_i(1-p_i)}{N_p}\right]\left[\frac{1}{q_i - r_i}\right]^2 + \left[\frac{r_i(1-r_i)}{N_r}\right] + \left[\frac{(q_i-p_i)}{(q_i-r_i)^2}\right]^2 + \left[\frac{q_i(1-q_i)}{N_q}\right]\left[\frac{p_i - r_i}{(q_i-r_i)^2}\right]^2 \qquad (3)$$

where:

n_p = number of pollen (# of embryos),

N_r = number of ovule (# of trees x 2), and

N_q = number of foreign pollen (# of trees in the natural stand x 2).

Inspection of (3) shows that at least moderate gene frequency differences between the orchard gene frequency r_i and the natural stand gene frequency q_i are needed to provide for a reasonably low variance of m_i. To obtain a greater level of precision, the number of trees can be increased, or m_i can be taken as an average over loci because its expected true value is the same at all loci.

This variance formula assumes no sex-differential fertility. This aforementioned factor would increase the Var(r) term by a factor approximately equal to the squared difference between the female (ovule) and male (orchard produced pollen) allele frequency. In addition, incomplete sampling of all orchard trees introduces additional variance (but no bias) as this is directly analogous to sex-differential fertility if this incomplete sampling causes errors of r_i. However, the extent of either of these factors is not quantifiable in the present study.

The minimum variance estimate of migration on an average over loci is the weighted average:

The minimum variance estimate of migration on an average over loci is the weighted average:

$$\hat{\bar{m}} = \left[\sum_{i=1}^{n} \frac{1}{V_{\hat{m}_i}}\right]^{-1}\left[\sum_{i=1}^{n} \frac{\hat{m}_i}{V_{\hat{m}_i}}\right] \qquad (4)$$

where:

n = number of loci studied.

This minimum variance estimate of m takes greater advantage of those loci showing greater differences of allele frequency between the orchard and natural stand, i.e., those loci providing more information about m.

The variance of $\bar{m}$ ($V_{\hat{\bar{m}}}$) is:

$$V(\hat{\bar{m}}) = \left[\sum_{i=1}^{n} \frac{1}{V_{\hat{m}_i}}\right]^{-1} \qquad (5)$$

The formulae for the minimum variance estimate of migration (4) and its variance (5) assume that migration estimates are approximately statistically independent among loci. More explicitly, the statistical covariance between migration estimates $\hat{m}_i$ and $\hat{m}_j$ at locus i and j, respectively, denoted Cov ($\hat{m}_i$, $\hat{m}_j$), equals Cov ($\hat{m}_i - \bar{m}$, $\hat{m}_j - \bar{m}$) + Var ($\bar{m} - m$) in expectation, where $\bar{m}$ is the migration (contamination) rate in the sample and m is the true migration rate. In our case, lack of linkage disequilibrium between loci makes Cov ($\hat{m}_i - \bar{m}$, $\hat{m}_j - \bar{m}$) equal zero, and the remaining term Var ($\bar{m} - m$) equals $m(1-m)/N$, which is small relative to the terms in (3) for small m and small ($q_i - r_i$), ($q_i - p_i$), and ($p_i - r_i$). Thus, (4) and (5) are suitable for larger populations with small to moderate gene frequency differences between subpopulations, little linkage disequilibrium relative to the total population, and small migration rates between subpopulations.

The advantage of this method of estimating contamination over that of Smith and Adams (1983) is that it does not rely upon there being a limited number of genotypes in the orchard, so that non-clonal orchards with more genotypes may be studied. In our situation, the large number of both clones and open-pollinated seedlings within the orchard made the generation of unique multilocus gametes by the natural stand at the loci surveyed highly unlikely. However, although our method uses data from all loci, it ignores any information provided by linkage disequilibrium between loci, with respect to the combined orchard-natural stand population, that the Smith and Adams method essentially relies upon. Such linkage disequilibrium, caused by finite population size, is expected to be relatively small in our case.

Results and Discussion

Prior to estimating the contamination percent for the studied blocks, the chi-square contingency test (Spiess, 1977) was conducted to assess the variability among each of the six blocks' and the three natural stands' gene pools. Outcrossing pollen and ovule gene pools were significantly ($P < 0.01$) heterogenous among blocks for five and six loci,

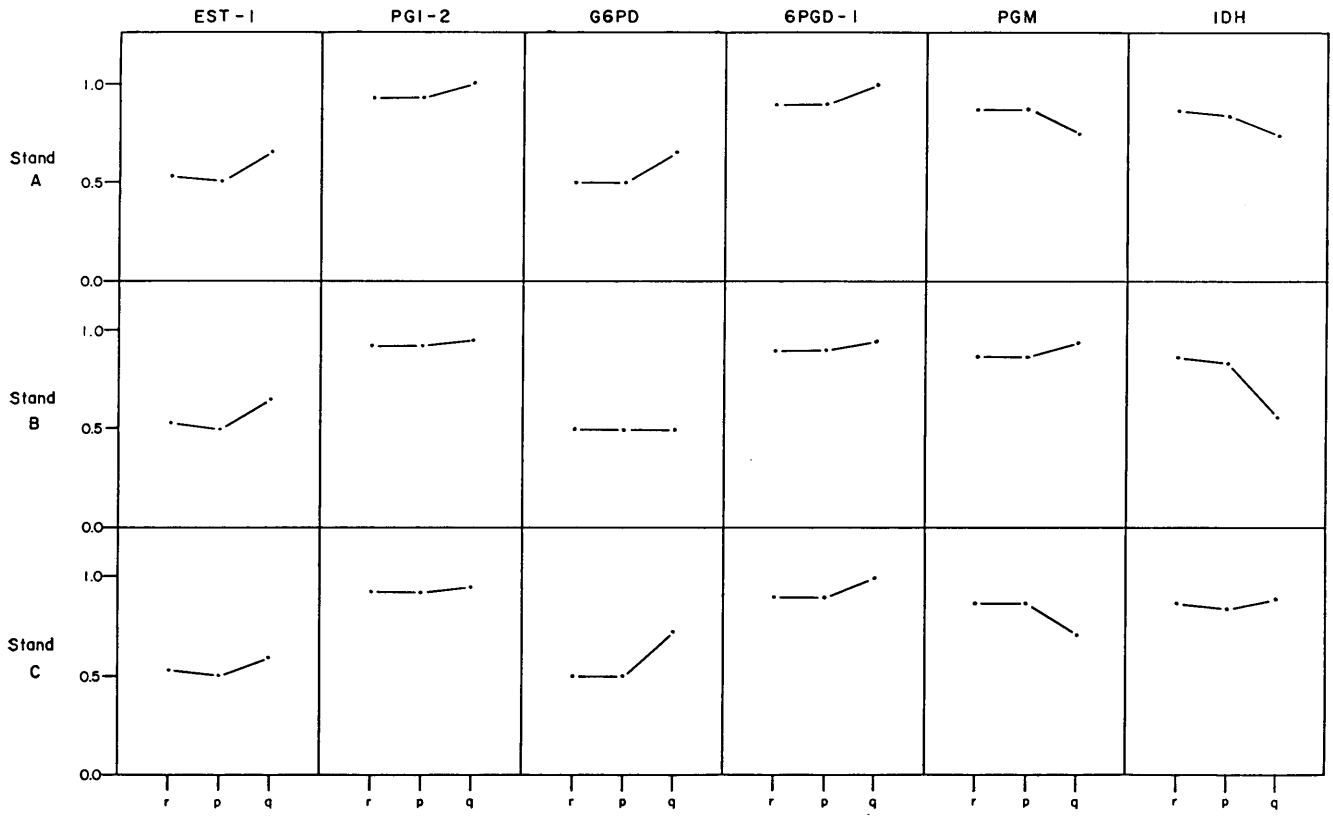

Fig. 3. — Plot of allele frequency (the most common allele) at six enzyme loci for the natural stands (A, B, C) and both of the pollen (p) and ovule (r) gene pools for samples obtained from block 6. N.B. 'q' = natural stand.

respectively *(Tables 1* and *2)*. The three natural stands (A, B, and C) also showed heterogenity *(Table 3)*. This heterogenity indicates that the contamination rate for each block can be estimated separately, and also that each of the three natural stands can be considered separately in turn as a potential contamination source.

Plots of most common allele frequencies of outcrossing pollen p_i and ovule gene pools r_i at six enzyme loci for one representative block (6) and the gene frequency q_i of the three natural stands (A, B, and C) are given in *Figure 3*. Contamination estimates, averaged over loci, for the 18 possible combinations (6 blocks × 3 stands) are presented in *Table 4*. The estimated proportion of contamination varied between −0.264 and 0.125 for the different block-stand combinations *(Table 4)*. Under the model assumptions, negative values can only be conceivably caused by statistical sampling error, and indeed none are significantly negative. This indicates that the variance approximation (3) is adequate, even though sex-differential fertility and incomplete sampling, which contribute additional compo-

Table 1. — Comparison of allelic frequencies in the outcrossing pollen pool among the studied six blocks.

Locus	Allele	Block						Chi-square †
		1	6	9	12	13	15	
EST-1	1	0.484	0.504	0.501	0.540	0.480	0.466	63.75**
	2	0.206	0.212	0.248	0.205	0.285	0.258	
	3	0.308	0.284	0.251	0.255	0.235	0.276	
PGI-2	1	0.933	0.927	0.922	0.925	0.924	0.937	8.61
	2	0.066	0.073	0.078	0.073	0.076	0.062	
	3	0.001	0.000	0.000	0.002	0.000	0.001	
G6PD	1	0.505	0.497	0.515	0.518	0.430	0.448	59.36**
	2	0.443	0.462	0.448	0.441	0.541	0.514	
	3	0.052	0.041	0.037	0.041	0.029	0.038	
6PGD-1	1	0.898	0.904	0.915	0.887	0.917	0.900	53.69**
	2	0.052	0.028	0.049	0.051	0.050	0.060	
	3	0.050	0.068	0.036	0.062	0.033	0.040	
PGM	1	0.885	0.883	0.913	0.896	0.955	0.878	119.24**
	2	0.081	0.052	0.061	0.055	0.031	0.054	
	3	0.034	0.065	0.026	0.049	0.014	0.068	
IDH	1	0.846	0.854	0.869	0.794	0.837	0.847	70.13**
	2	0.096	0.072	0.082	0.089	0.091	0.095	
	3	0.058	0.074	0.049	0.117	0.072	0.058	
N		1186	1663	762	1519	1524	2192	

† Chi-square d.f. = (r-1) (c-1) = (3-1) (6-1) = 10

** Significant at 0.01 probability level.

Table 2. — Comparison of allelic frequencies in the ovule pool among the studied six blocks.

Locus	Allele	Block						Chi-square †
		1	6	9	12	13	15	
EST-1	1	0.573	0.536	0.617	0.530	0.490	0.557	115.78**
	2	0.250	0.196	0.167	0.250	0.312	0.236	
	3	0.177	0.268	0.216	0.220	0.198	0.207	
PGI-2	1	0.937	0.937	0.900	0.910	0.927	0.943	80.78**
	2	0.063	0.063	0.100	0.090	0.073	0.050	
	3	0.000	0.000	0.000	0.000	0.000	0.007	
G6PD	1	0.469	0.509	0.517	0.490	0.437	0.436	103.23**
	2	0.458	0.464	0.433	0.470	0.542	0.528	
	3	0.073	0.027	0.050	0.040	0.021	0.036	
6PGD-1	1	0.906	0.893	0.933	0.890	0.906	0.879	102.00**
	2	0.042	0.027	0.067	0.050	0.052	0.064	
	3	0.052	0.080	0.000	0.060	0.042	0.057	
PGM	1	0.896	0.884	0.850	0.900	0.948	0.864	231.74**
	2	0.083	0.036	0.133	0.060	0.031	0.072	
	3	0.021	0.080	0.017	0.040	0.021	0.064	
IDH	1	0.854	0.876	0.800	0.800	0.792	0.886	139.54**
	2	0.094	0.062	0.100	0.090	0.094	0.071	
	3	0.052	0.062	0.100	0.110	0.114	0.043	
N		48	56	30	50	48	70	

† Chi-square d.f. = (r-1) (c-1) = (3-1) (6-1) = 10

** Significant at 0.01 probability level.

nents of error in the estimate are ignored. The average stand and block estimated contamination varied between −0.006 to 0.005 and −0.226 to 0.066, respectively, with an overall estimated contamination of 0.002 ± 0.057 *(Table 4)*. Thus, the maximum contamination of the orchard by outside pollen is about 6 percent, and it may be as low as zero.

This relatively small confidence interval of ±0.057 for the contamination rate has resulted, despite there existing only small gene frequency differences between the orchard and natural stands *(Table 1, 2, 3)*. Both tle large number of zygotes and the many loci used in this combined overall estimate have been responsible for this relatively small interval.

Since 64 out of the 80 orchard genotypes were sampled across the six blocks, it is conceivable that some of the contamination may have actually been due to unsampled clones that minimiced the natural stand gene frequency. Like arguments hold for sex-differential fertility, wherein the unmeasured male component may have mimicked the natural stand. However, there is no *a priori* basis to expect a systematic mimicking over loci. It would be desirable, but difficult, to more than visually estimate the male cone production, and hence correct for sex-differential fertility.

The estimated contamination rate of 6 percent for this year (1983) is lower than the 15 percent reported for the same orchard for 1976 and 78 under the same cooling treatment (Fashler and Devitt, 1980) and the estimates that ranged from 5—15 percent for 1982 under natural conditions (no cooling) for the same orchard (Clare, 1982). Both of these studies were based upon pollen counts per unit area from several pollen traps located on a transect between the natural stand "C" and the orchard. These methods used the pollen counts per unit area and distance from the putative pollen source (natural stand) to develop linear regression models. The inferred regression models in turn were used to predict the expected pollen count in different locations around as well as inside the orchard.

However, a critical statistical assumption was violated with this approach, in that the developed regression models are strictly valid only for prediction between the sampling points range, and not beyond these points (Steel and Torrie, 1980), i.e., inside the orchard. One explanation for our lower estimate of contamination is that wind speed drops within the orchard and thus pollen from outside sources may not penetrate the orchard at the rate predicted by regression model. A comparison of estimates using genetic markers vs. pollen traps in the same year is needed to determine if there is a substantial bias of migration estimates obtained via the pollen trap method.

The reported low contamination rate in this study could also be explained by other biological and physical factors that operated only during 1983. These include the water-spray cooling treatment that was applied in 1983 but not in 1982, and the massive orchard reproductive (male and female) cone production in 1983 which may have diluted the frequency of foreign pollen, in addition to the presence of a buffer zone in the northern side of the orchard (facing the prevailing wind direction) *(Figure 1)*. Regardless, the utility of using allozyme markers in a population genetic context for direct estimates of pollen contamination has been demonstrated in this study.

Table 3. — Comparison of allelic frequencies among the three natural stands.

Locus	Allele	stand A	stand B	stand C	Chi-square†
EST-1	1	0.650	0.660	0.590	10.91(4)*
	2	0.200	0.170	0.090	
	3	0.150	0.170	0.320	
PGI-2	1	1.000	0.920	0.950	6.98(2)*
	2	0.000	0.080	0.050	
G6PD	1	0.650	0.500	0.720	11.77(4)*
	2	0.300	0.460	0.230	
	3	0.050	0.040	0.050	
6PGD-1	1	1.000	0.960	1.000	7.11(2)*
	2	0.000	0.040	0.000	
PGM	1	0.750	0.960	0.720	27.22(4)**
	2	0.200	0.040	0.140	
	3	0.050	0.000	0.140	
IDH	1	0.750	0.580	0.900	26.14(4)**
	2	0.100	0.210	0.050	
	3	0.150	0.210	0.050	
N		20	24	22	

† Chi-square d.f. = (r-1) (c-1)

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

Table 4. — Estimates of contamination and their 95% confidence intervals for every block-natural stand combination.

Block	Stand A	Stand B	Stand C	Average
1	0.024 ± 0.277	−0.020 ± 0.248	0.047 ± 0.263	0.015 ± 0.151
6	−0.019 ± 0.248	0.058 ± 0.215	−0.018 ± 0.256	0.013 ± 0.137
9	−0.209 ± 0.438	−0.264 ± 0.367	−0.198 ± 0.372	−0.226 ± 0.224
12	0.058 ± 0.270	0.016 ± 0.304	0.035 ± 0.240	0.038 ± 0.154
13	−0.031 ± 0.175	−0.151 ± 0.284	−0.015 ± 0.164	−0.042 ± 0.110
15	0.039 ± 0.232	0.125 ± 0.196	0.013 ± 0.223	0.066 ± 0.124
Average	−0.006 ± 0.100	0.005 ± 0.102	−0.006 ± 0.094	0.002 ± 0.057

Acknowledgements

This project was supported by the British Columbia Ministry of Forests, Research Branch, Section 88 nos. JS-02-016 and KS-02-012; by Pacific Forest Products Ltd., Victoria, B. C.; by a NSERC (Canada) grant to Fred R. Ganders (U.B.C.); and by a Nat. Inst. of Health (USA) grant to KR. The authors are indebted to M. Carlson for critical comments, J. Parkinson for technical assistant, C. Cook for the helicopter cone collection, and L. Irvine for preparing the figures. The use of the computing facility of the Faculty of Forestry, University of British Columbia is highly appreciated.

Literature Cited

Bridgwater, F. E. and Trew, I. F.: Supplemental mass pollination. In: E. C. Franklin (ed.). Pollen Management Handbook. U.S.D.A. Agriculture Handbook 587. Washington, D. C. pp. 15—19 (1981). — Clare, L. R.: Assessment of pollen contamination in Pacific Forest Products' high-elevation seed orchard. BSF thesis, Faculty of Forestry, University of British Columbia, Vancouver (1982). — Denison, N. P.: A review of aspects of tree breeding in the Republic of South Africa. In: J. Burley and D. G. Nikles (eds.). Selection and breeding to improve some tropical conifers. Oxford. Comm. For. Inst. 2: 277—284 (1973). — El-Kassaby, Y. A., Yeh, F. C. and Sziklai, O.: Inheritance of allozyme variants in coastal Douglas-fir *(Pseudotsuga menziesii* var *menziesii)*. Can. J. Genet. Cytol. 24: 325—335 (1982). — Fashler, A. M. K. and Devitt, W. J. B.: A practical solution to Douglas-fir seed orchard pollen contamination. For. Chron. 56: 237—241 (1980). — Friedman, S. T. and Adams, W. T.: Genetic efficiency in loblolly pine seed orchards. In: Proc. 10th South. For. Tree Improv. Conf., Blacksburg, VA. pp. 213—224 (1981). — Hadders, G.: Kontroll av inkorsningen i en tallplantage. Foren. Skogstradsforadlings Arsb. (1973). Uppsala (1972). — Ritland, K. and El-Kassaby, Y. A.: The nature of inbreeding in a seed orchard of Douglas-fir as shown by an efficient multilocus model. Theor. Appl. Genet. 71: 375—384 (1985). — Sarvas, R.: Establishment and registration of seed orchards. Folia For. Fenn 89 (1970). — Silen, R. R.: Effect of altitude on factors of pollen contamination of Douglas-fir seed orchards. J. For. 61 :281—283 (1963). — Silen, R. R. and G. Keane: Cooling a Douglas-fir seed orchard to avoid pollen contamination. U.S.D.A., For. Serv., Res. Note PNW-

101, 10 pp., (1969). — SMITH, D. B. and ADAMS, W. T.: Measuring pollen contamination in clonal seed orchards with the aid of genetic markers. In: Proc. 17th South. For. Tree Improv. Conf., Athens, GA. pp. 69—77 (1983). — SPIESS, E. B.: Genes in populations. John Wiley and Sons, Inc., N. Y. (1977). — SQUILLACE, A. E.: Effectiveness of 400-foot isolation around a slash pine seed orchard. J. For. 65: 823—824 (1967). — SQUILLACE, A. E. and LONG, E. M.: Proportion of pollen from non-orchard sources. In: E. C. FRANKLIN (ed.). Pollen Management Handbook. U.S.D.A. Agriculture Handbook 587. Washington, D.C. pp. 15—19 (1981). — STRAND, L.: Pollen dispersal. Silvae Genet. 6: 129—136 (1957). — STEEL, R. G. D. and TORRIE, J. H.: Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill Book Company, N.Y. (1980). — WERNER, M.: Location, establishment and management of seed orchards. In: R. FAULKNER (ed.). Seed Orchards. For. Comm. London Bull. No. 54, 49—57 (1975). — WOESSNER, R. A. and FRANKLIN, E. C.: Continued reliance on wind-pollinated southern pine seed orchards, is it reasonable? In: Proc. 12th South. For. Tree Improv. Conf. pp. 64—73 (1973). — WRIGHT, J. W.: Pollen-dispersion studies: Some practical applications. J. For. 51: 114—118 (1953).

Genetic Parameters and Gains expected from Selection in Pinus caribaea var. hondurensis in Northern Queensland, Australia

By C. A. DEAN[1]), P. P. COTTERILL[1]) and R. L. EISEMANN[2])

(Received 16th December 1985)

Summary

Estimates of individual heritabilities and genetic and phenotypic correlations are presented for growth and form traits measured across five polycross or open-pollinated progeny tests of *P. caribaea* var. *hondurensis* in northern Queensland, Australia. The growth traits were measured at 4½, 7½ and 10½ years after planting while the form traits were assessed visually at 7½ years. In the case of the 7½ year measurements, stem height had moderate to high individual heritabilities (values ranging from 0.16 to 0.41 across the five progeny tests), stem diameter (0.33 to 0.73) and stem straightnes (0.29 to 0.49) had high heritabilities, and branch diameter (0.14 to 0.38) and branch angle (0.16 to 0.37) moderate to high heritabilities. Stem diameter had adverse genetic correlations with stem straightness (−0.02 to −0.45) and branch diameter (−0.49 to −0.88). Stem height had adverse genetic correlations with straightness in all but one of the progeny tests (0.18 to −0.45) and strong adverse correlations with branch diameter in all tests (−0.24 to −0.79).

Genetic gains which might be expected under such conditions of adverse correlation were examined by placing weightings on height, stem diameter, stem straightness and branch diameter in selection indices. Results suggest that it is not possible to achieve substantial simultaneous improvement in both growth and form of *P. caribaea* var. *hondurensis* by selection within the species. Options, including hybridisation, are suggested which may overcome this limitation.

Key words: *Pinus caribaea*, heritability, genetic correlation, genetic gain, growth, form.

Zusammenfassung

Für die erhobenen Wachstumswerte und Formmerkmale aus 5 Polycross- oder frei abgeblühten Nachkommenschaften von *Pinus caribaea* var. *hondurensis* im nördlichen Queensland in Australien werden Schätzungen der individuellen Heritabilitäten und der genotypischen sowie phänotypischen Korrelationen vorgebracht. Die Wachstumswerte wurden 4½, 7½ und 10½ Jahre nach der Pflanzung ermittelt, und die Formmerkmale nach 7½ Jahren bonitiert. Bei den Messungen nach 7½ Jahren hatte die Stammhöhe mäßige bis hohe Heritabilitäten (Werte von 0,16—0,41 über die 5 Nachkommenschaften hinweg). Der Stammdurchmesser (0,33 bis 0,73) und die Geradschäftigkeit (0,29 bis 0,49) hatten hohe, der Astdurchmesser (0,14 bis 0,38) und der Astwinkel (0,16 bis 0,37), mäßige bis hohe Heritabilitäten. Der Stammdurchmesser zeigte nachteilige genetische Korrelationen mit der Geradschäftigkeit (−0,02 bis −0,45) und dem Astdurchmesser (−0,49 bis −0,88). Die Stammhöhe hatte, bei allen Nachkommenschaften bis auf eine, nachteilige genetische Korrelationen mit der Geradschäftigkeit (0,18 bis −0,45) und bei allen Tests starke nachteilige Korrelationen mit dem Astdurchmesser (0,24 bis —0,79).

Genetische Gewinne, die unter solchen Bedingungen nachteiliger Korrelation erwartet werden könnten, wurden durch die Gewichtung von Höhe, Stammdurchmesser, Geradschäftigkeit und Astdurchmesser in Selektionsindizes untersucht. Die Resultate zeigen, daß es nicht möglich ist, eine substantielle und gleichzeitige Verbesserung sowohl beim Wachstum, als auch bei der Form von *Pinus caribaea* var. *hondurensis* durch Selektion innerhalb der Art zu erreichen. Es werden Möglichkeiten einschließlich der Hybridisierung vorgeschlagen, um diese Beschränkung zu überwinden.

Introduction

Pinus caribaea MORELET has become an important species in exotic plantations in many tropical and subtropical regions, with an estimated global planting rate of about 100,000ha per annum (NIKLES, 1979). The bulk of these plantations comprise var. *hondurensis* BARRETT and GOLFARI which is generally preferred to var. *bahamensis* and var. *caribaea*. Historically, the most widely used provenance of var. *hondurensis* has been that from the Mountain Pine Ridge area of Belize (NIKLES, 1979). In Queensland *P. caribaea* var. *hondurensis* has been planted for 35 years and plantations are made up almost entirely of trees from this Mountain Pine Ridge provenance.

Breeding programs to improve *P. caribaea* var. *hondurensis* have been in progress for over two decades in Queensland. Basic genetic information is not available, however, to assist in formulating the most effective breeding strategies. The only documented estimates of genetic parameters for *P. caribaea* appear to be for growth and form traits in a progeny trial in Puerto Rico (LEDIG and WHITMORE, 1981) and some limited estimates for early growth and foxtails from Brazil (KAGEYAMA *et al.*, 1983; KAGEYAMA *et al.*, 1984).

[1]) Division of Forest Research, CSIRO, The Cunningham Laboratory, 306 Carmody Road, St. Lucia, 4067 (Australia).

[2]) Queensland Forestry Department, Brisbane. (Now at: Queensland Wheat Research Institute, P.O. Box 5282, Toowoomba, 4350 Australia.)