

FIA-FSP Project Y062110
Executive Summary

Evaluating the Protocol for Quantifying Pollen Contamination on the Genetic Worth of
Conifer Seed Orchards.

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Project Purpose

Since all conifers are wind pollinated, orchards located within the natural range of the species are susceptible to non-orchard sources of pollen (contamination). Contaminate pollen is considered to have a negative effect on both the orchard's estimated genetic worth of the improved trait (for example growth) and the adaptive potential of seed orchard progeny.

Our current protocol for assessing the contribution of pollen contamination uses pollen monitoring technique. Both the period of pollen shed and the density of pollen clouds for both orchard and non-orchard sources are measured. Depending on the orchard site, the genetic worth of a specific orchard parent is reduced by the proportion of total contaminate pollen to orchard pollen.

The species selected for this study is coastal Douglas-fir since most of the orchards are located on eastern Vancouver Island. The assumption made with the current protocol for estimating the gamete contribution of contaminate pollen is that contaminate pollen is related to its calculated proportion in the orchard pollen cloud as measured by pollen monitoring technique. Until recently, we had no better way to assess pollen contamination. Now paternity analyses, using molecular technique has been successfully developed for seed orchard applications. While it is possible to identify both orchard and non-orchard sources of pollen parents using DNA fingerprinting, it is unlikely we will adopt this new technique for routine gamete contribution. It will, however, allow us to assess the accuracy of our current pollen monitoring technique and provide orchard managers with confidence that the annual calculations of a seed lot's genetic worth are reliable. Furthermore, since a seedlot's genetic worth is now incorporated directly into timber supply analysis, future estimates of wood production will also be more reliable.

Project Start Date

This project began in 2004 (Y051110) and will continue into 2006/07 (Y073110). This report summarizes the results to date including the second year (Y062110).

In year one (Y051110), estimates of pollen contamination from pollen monitoring and DNA paternity analyses were far from similar. For example, mean contamination at the Bowser and WFP sites were 47% and 100%, respectively, from pollen monitoring data and 2.4% and 1.3%, respectively from the DNA paternity analyses. Why these estimates were so far apart became a principal objective for the second year of this study.

For pollen monitoring, we held on-site training sessions for identifying and counting pollen. Previously, orchard technicians counted pollen with low power (x40) dissecting microscopes which were not able to distinguish either the size or fine sculpturing detail of species of pollen (western hemlock, grand fir and Sitka spruce) shedding coincidentally with Douglas fir. We also improved the estimates of contaminate pollen from paternity analyses based on the proportion of unique genotypes in both the orchard and surrounding stand populations.

Methodology

A. Pollen Monitoring

The extent of pollen contamination is measured over the duration of receptivity of each orchard clone. Total orchard contaminate pollen is then calculated as the ratio of total contaminate pollen to total orchard pollen (x100%) over the receptivity period of the orchard.

B. Paternity Analyses

The previously identified hypervariable region of the Douglas-fir chloroplast genome was used to establish baseline genotypes of orchard clones and seed resulting from wind-pollinations in the orchard. Since the chloroplast DNA (cpDNA) is inherited paternally in conifers, i.e., through the pollen, it was an ideal genetic marker to determine the pollen parent of a wind-pollinated seed.

Total DNA from all samples was then amplified with chloroplast-specific primers using the polymerase chain reaction (PCR). Genotypes were assigned to each embryo analyzed and classified as either being sired by an orchard male or, if the paternal genotype was not present in the orchard, as sired by a contaminant male.

This analysis still leaves questions about the origin of genotypes that would be common to both the orchard and background sources of pollen. To answer this question, we sampled 100 individual trees (genotypes) from the background stands surrounding both the Bowser and WFP orchard sites. All 200 trees were genotyped and a number of unique genotypes (occurring only in the surrounding stands (i.e., non-orchard sources) were identified. If we assume that the sampling procedure (100 trees) represents the population of surrounding stands, then the ratio of percent unique genotypes (i.e., arising from back ground or contaminate pollen) in the orchard to the percent of unique genotypes found only in the background population should be a more accurate estimate of orchard pollen contamination.

Project Scope

Two contrasting orchard sites were selected: one orchard located on lower Vancouver Island (Western Forests Products) in Saanichton and the other located in central Vancouver Island (Ministry of Forests) at Bowser. Estimates of contamination by both pollen monitoring and DNA analyses was completed on clones selected over the entire receptivity of the orchard.

To determine the proportion of alleles in the contaminate stands surrounding each orchard site, a total of 102 mature Douglas-fir trees in the vicinity of the WFP orchard in Saanichton and 100 mature Douglas-fir trees in the background of the MoF orchard at Bowser were genotyped using a highly-variable chloroplast DNA marker. There was significant overlap in the molecular fingerprinting pattern between the outside-orchard

trees and within-orchard trees. However, there were a number of genotypes in both background populations that are not present in the orchards.

This made it possible to determine pollen contamination if certain assumptions were made: 1) The distribution of the unique genotypes in the background is random and sampling did not bias their chance of detection; 2) we can assume that the proportion of sampled, unique genotypes compared to common background genotypes is the same in the surrounding trees as it is in the pollen pool of the orchard.

Results

The mean levels of contamination in the two coastal Douglas-fir seed orchards in 2005 was, in general, lower than 2004 but were significantly closer for the two methods. Estimates of pollen contamination by pollen monitoring still yielded the higher estimates but they were much closer than last year. Table 1 summarizes the levels of contamination calculated from pollen monitoring and determined from DNA paternity analyses.

Table 1: Levels of 2005 Douglas-fir pollen contamination in Western Forests Products, Saanich Seed Orchard (166) and the Ministry of Forests, Bowser Seed Orchard (162) using both pollen monitoring and DNA data.

Pollen Monitoring	WFP	MoF
%Orchard	5.5%	21.5%
%Clones	2.2%	20.1%
DNA Paternity Analyses		
Number Clones Surveyed	17	14
Number of Ramets	35	28
Number Ramets Contaminated	17	19
Range of Contamination	0-21.2%	0-15.6%
Mean Contamination	2.9%	5.8%

The estimates of contamination (Table 1) are based on the identification of unique genotypes that do not occur in the orchard population. However, paternity analysis still had some questions about the origin of genotypes that would be common to both the orchard and background sources of pollen. To answer this question, we sampled about 100 individual trees (genotypes) from the background stands surrounding both the Bowser and WFP orchard sites. All 200 trees were genotyped and a number of unique genotypes (occurring only in the surrounding stands (i.e., non-orchard sources) were identified. If we assume that the sampling procedure (100 trees) represents the population of surrounding stands, then the ratio of percent unique genotypes (i.e., arising from background or contaminate pollen) in the orchard to the percent of unique genotypes found only in the background population should be a more accurate estimate of orchard pollen contamination.

Based on the genotyping work of surrounding stands (2005), we found 14 out of 102 genotypes in the background stands surrounding WFP were unique, i.e., not found in the orchard. This presents a proportion of 13.7%. At the Bowser seed orchard, 9 out of 100 genotypes were unique (9%). If we assume that these unique genotypes represent the

contaminate population, then the ratios of unique genotypes in the orchard to surrounding stands is a more accurate estimate of contamination. For the 2005 molecular analysis, 53 out of the 899 seeds analyzed from Bowser (Table 5) and 32 seeds out of 1051 seeds analyzed from WFP (Table 8) orchard could be assigned to the parentage of the unique genotypes (i.e., non-orchard sources). However, from the unique genotypes identified from the 2005 analyses of surrounding stands (Table 2), only 9 of 100 from Bowser and 14 of 102 from WFP were identified as unique. Since these seeds represent only 9.0% and 13.7% of the total potential contamination, the estimates of contamination values need to be extrapolated upwards as shown below:

Bowser:

19 seeds represent contaminant seeds from 9% of all background parents

If X is the total number of contaminate seeds, then

$$X = 19 \text{ seeds} / 9\% \times 100 = 211 \text{ seeds}$$

$$\text{Total contamination \% is then: } 211 \text{ seeds} / 899 \text{ seeds} \times 100 = \mathbf{23.5\%}$$

WFP:

14 seeds represent contaminant seeds from 13.7% of all background parents

If X is the total number of contaminate seeds, then

$$X = 14 \text{ seeds} / 13.7\% \times 100 = 102 \text{ seeds}$$

$$\text{Total contamination \% is then: } 102 \text{ seeds} / 1051 \text{ seeds} \times 100 = \mathbf{9.7\%}$$

Estimates obtained for this year's seed crops are remarkably similar to those obtained last year (7.3% vs. 9.7% for WFP and 23.2% vs. 23.5% for Bowser, for the 2004 and 2005 crop, respectively). Levels of contamination based on pollen monitoring and molecular techniques produced very different results in the first year of this study. Pollen monitoring grossly over estimated contamination while PCR technique under estimated contamination. Both these issues were addressed in this year's work.

First, contamination estimates from pollen monitoring suffered from poor pollen identification and counting procedures. In general, counts of Douglas-fir pollen also included other species that shed coincidentally. This was easily rectified by providing technicians with on-site training in pollen identification. Morphology (size, exine sculpturing) of pollen shedding with Douglas-fir (western hemlock, grand fir and Sitka spruce) can be distinguished with microscopes capable of x100 resolution. Compound microscopes replaced the lower powered dissecting microscopes in use by all orchards. Also the sampling procedure for counting slides at WFP was modified to provide better estimates of counts over the entire slide.

DNA paternity analyses provided much better estimates of contamination which were improved even further by sampling unique genotypes in the surrounding stands of both orchard sites. The difference between estimates of contamination from pollen monitoring and paternity analyses in 2004 was about 10 fold. However, now that orchard technicians can properly identify Douglas-fir pollen, the estimates for contamination in 2005 for WFP and Bowser were 5.5% and 21.5% from pollen monitoring and 9.7% and 23.5% for paternity analyses, respectively.

Conclusions and Management Implications

It is clear that the pollen monitoring technique applied in 2004 failed to provide reliable estimates of pollen contamination in the two coastal Douglas-fir seed orchard sampled. However, results from 2005 are very encouraging. Estimates of contamination differed by only a few percent and are well within the errors we can tolerate. Pollen cloud density values for both 2004 and 2005 were similar so we can not expect the same results when a heavy pollen cloud occurs. However, we can feel confident that both procedures now provide a more reliable estimate of contamination and pollen monitoring technique can be used with more confidences to adjust levels of male gamete contribution in the annual seed lot rating procedures. Furthermore, since the genetic worth of seedlot is now incorporated directly into timber supply analysis, future estimates of wood production would have a higher associated error.

Contact Information

A detailed summary of the two years results is available and additional information regarding the estimates of pollen contamination in coastal Douglas-fir orchards will be provided by

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