

FSP 2006/07
Final Technical Report

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

Project Y073110

Joe Webber and Michael Stoehr

Abstract

Seed orchards are typically located in southern, warmer climates which provide a natural inductive effect for improved flowering. Since conifers are wind pollinated and the periods of reproductive development for indigenous stands and orchard clones overlap, non-orchard sources of pollen (contamination) can sire a significant number of female flowers in the orchard. This has been identified as a problem in coastal Douglas-fir.

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 GW of a specific orchard parent is reduced by the proportion of total contaminate pollen to orchard pollen.

The assumption we are making with this approach, is that gamete contribution of contaminate pollen is related to its calculated proportion as measured by pollen monitoring technique. With no reliable method to test our assumptions, errors arising from our assumptions and its impact on GW can also carry through to every stage of Timber Supply Analyses. Until recently, we had no better way to assess pollen contamination. Now DNA paternity analysis provides us with a robust technique for assessing these assumptions.

In year-one of this project (2004), contamination levels in two contrasting coastal Douglas-fir seed orchards (Saanich and Bowser, Vancouver Island) using the current pollen monitoring protocol were 100% and 47%, respectively. Estimates of contamination from DNA paternity analysis for the same two seed lots were 1.3% and 2.4%, respectively.

In year two of this study (2005), we focused on why the estimates of pollen contamination from these two techniques were so different. First, the pollen monitoring protocol was improved (better microscopes for counting and training sessions in identifying pollen) and, second we genotyped background trees that surrounded each orchard site to get a better estimate of the proportion of unique DNA fingerprints that was found only in the background population. Applying this new data to the 2004 crop, estimates of pollen contamination from DNA paternity analyses for the Saanich and Bowser orchards were raised to 8.5% and 23.2%, respectively.

The improved pollen monitoring and DNA paternity analyses were applied to the 2005 and 2006 crop years. Estimates of contamination levels were much closer and varied by only a few percentage points. We now feel confident that pollen monitoring can produce reliable estimates of contamination and work will continue to standardize the technique across all orchards.

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Introduction

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.

To register seed for use on crown land, orchard managers must calculate the weighted gamete contribution (both male and female) for each seedlot (Woods et al. 1996, Stoehr et al. 2004, Woods 2004). Where contamination levels are high (>50%), a substantial reductions in the orchard's genetic worth would occur. For example, a seedlot rated at a genetic worth of 20% and exposed to 50% contamination would lose 5% of the seedlot's GW contributed by the pollen resulting in a net reduction of the seedlot's GW to 15%. Since a seedlot's GW is now incorporated in timber supply analyses, even a 5% loss in growth potential would also equate to a 5% loss in wood volume or more if the planting stock is also poorly adapted.

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 from contamination. It will, however, allow us to assess the accuracy of our current pollen monitoring technique.

It was our contention that pollen monitoring can reasonably reflect annual levels of contaminate pollen. However, the technique was not being applied consistently across all orchards. The methods to capture and count pollen grains varied among the orchards. Consequently, a technical audit was required to determine where significant errors were occurring.

Objectives for this three year project focused on the inconsistencies in how pollen is trapped, identified and expressed as pollen cloud density and how estimates of contamination were calculated using paternity analyses. Specific objectives were as follows:

- to assess pollen monitoring technique at each of two orchard sites on Vancouver Island, and,

- to calculate levels of pollen contamination using both pollen monitoring and DNA paternity analyses to the 2004, 2005 and 2006 seed crops.

Methods

A. Pollen Monitoring

The current protocol for rating seed orchard crops is described by Woods et al (1996). Levels of contamination are estimated by comparing the extent and duration of pollen catch from pollen monitoring stations from within the orchard (ORC) to regional stations located at a distance from the orchard (REG). The extent of pollen contamination is measured over the duration of receptivity of each specific orchard.

The receptivity period of each orchard was determined by detailed phenological assessments. Reproductive development of each crop tree was rated using a numerical assessment. A tree was scored as receptivity when 20% of the flowers were receptive to pollen (see Webber and Painter 1996) and past receptivity when 80% of the flowers were no longer receptive to pollen (Woods et al. 1996). For calculating levels of contamination, the period between 20% of the orchard clones reaching seed-cone receptivity and 80% of the clones passing seed-cone receptivity is used.

The ministry standard for monitoring pollen cloud density uses 7-day pollen recorders (see Webber and Painter 1987) and expresses pollen cloud density as grains/mm²/24h. However, only the ministry orchard (Bowser) uses the same technique for monitoring orchard and regional pollen catch. All other orchards use a microscope slide attached to a platform and weather vane assembly. Slides are changed daily and pollen catch is also expressed as grains/mm²/24h.

Pollen catch efficacy of this two monitoring techniques are not necessarily the same. To account for these differences, Woods et al (1996) introduced an orchard adjustment factor (OAF). The OAF is calculated for both early and late pollen catch where orchard pollen has not begun shedding or has completed shedding. Preferably, early pollen catches is used since it is difficult to be certain that orchard pollen has completely shed. The OAF is then multiplied by the REG mean daily pollen catch summed over the receptivity period of the orchard. When developing the OAF, it was assumed that the pollen catch efficiency was greater than the slide capture efficiency and by adjusting the REG total pollen catch would provide more realistic estimates of pollen contamination.

The percent pollen contamination is then calculated as the ratio of total contaminate pollen to total orchard pollen (adjusted REG/ORCx100%) over the receptivity period of the orchard.

B. Paternity Analyses

The previously identified hypervariable region of the Douglas-fir chloroplast genome (Stoehr et al. 1998) 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.

Vegetative tissue (buds) was used to genotype all orchard clones and embryo tissue to determine the origin of the male parent. Total DNA was extracted using liquid nitrogen followed by chloroform extraction for buds and Chelex-100 (BioRad) for embryos (Stoehr and Newton 2002). Total DNA from all samples was then amplified with chloroplast-specific primers using the polymerase chain reaction (PCR), according to Nelson et al. (2003). If amplified DNA bands on gels could not be unambiguously distinguished and clones separated solely on size, amplified DNA products were digested with restriction enzymes to elucidate sequence polymorphisms (Nelson et al. 2003). Restricted DNA was size-fractionated on 10% non-denaturing polyacrylamide gels, followed by ethidium bromide staining. Gels were scored using digital photography and Scion Image Analysis package. Then, multi-gene 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. In the event that restricted DNA analyses did not yield informative separation, the amplified DNA samples were sequenced in a commercial laboratory.

However, the 2004 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, in 2005 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 background or contaminate pollen only) 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.

C. Experimental Design

Two contrasting orchard sites were selected for evaluating the current protocol for calculating pollen contamination. One orchard is located on lower Vancouver Island (Western Forests Products) in Saanichton and the other is located in central Vancouver Island (Ministry of Forests) at Bowser. Based on provenance trials, contamination on the peninsula site was rated with a low, negative (-15%) breeding value with a high risk of maladaptation. More recently the negative breeding value of lower Vancouver Island has been raised to neutral (0%).

The central Island site at Bowser has both a neutral (0%) breeding value and a neutral risk of maladaptation. The two orchard sites also offer contrasting periods of contamination. On the peninsula, natural stand pollen shed normally occurs in the early stages of orchard receptivity whereas at the Bowser site pollen contamination can occur throughout the receptivity stage of the orchard.

Estimates of contamination by both pollen monitoring and DNA analyses was completed on clones selected over the entire receptivity of the orchard. For the Saanich site, contamination was expected to be more prevalent in the early stages of orchard receptivity whereas the north Island site at Bowser, contamination was expected to continue throughout the receptivity period of the orchard. For the Saanich site, sampling was more intense in the early stages of receptivity and more evenly distributed at Bowser. For Bowser, two ramets from each of 3 clones in each of 4 periods over the receptivity period of the orchard were selected. At the Saanich site, two ramets from each of 6 clones in the early, four in the mid and 2 in the late stages of orchard receptivity were selected. The final selection of clones varied somewhat over the three

year period because of variation in the annual cone crop. In the fall, four cones from each selected tree were collected, hand extracted, and filled seed per cone determined using x-ray analysis.

From collections made in 2005, we determined the proportion of unique 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 MoFR 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 were not present in the orchards. This made it possible to further refine the estimates of 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; and,
- 2) we can assume that the proportion of sampled, unique genotypes compared to common background genotypes is the same in the standing trees in the surrounding stands as it is in the pollen pool of the orchard.

For the 2005 molecular analysis, 53 out of the 899 seeds analyzed from Bowser and 32 seeds out of 1051 seeds analyzed from WFP 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 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

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

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

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

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

Estimates obtained for 2005 were remarkably similar to those obtained in 2004 (7.3% vs. 9.7% for WFP and 23.2% vs. 23.5% for Bowser, for the 2004 and 2005 crop, respectively) when the values were adjusted for the ratio of unique surround genotypes within the orchards (Table 4).

Results

A. 2004

In the first year of this project (Y051110), we compared orchard methods for monitoring pollen cloud density in two contrasting orchard sites on Vancouver Island (Saanich

peninsula and Bowser). Calculated levels of pollen contamination were then compared to the more robust DNA paternity analyses.

Table 1 shows the result based on pollen counts supplied by each of the two orchards. Variation between estimates of contamination from pollen monitoring and paternity analyses was extreme. For example, mean contamination at the Bowser and WFP sites were 47% and 100%, respectively, from pollen monitoring data and 2.1% and 1.3%, respectively from the DNA paternity analyses.

Estimates of contamination by clone also showed a wide discrepancy between pollen monitoring and DNA data. Tables 2 and 3 compared estimates of contamination for each selected clone in Bowser (Table 2) and WFP (Table 3).

The results for 2004 confirmed that estimates of contamination using pollen monitoring were not reliable. We also knew that DNA paternity analysis was underestimating actual levels of contamination. Restricted DNA analysis has its own limitations. There are certain genotypes that are common to both the orchard and contaminate stands that could not be unambiguously identified.

B. 2005

In the second year (Y062110) considerable improvement was made in estimates of contamination by pollen monitoring and paternity analysis. First we sampled 102 trees surrounding WFP orchard in Saanich and 100 trees surrounding the MoFR orchard at Bowser. Second, we identified several significant errors in the procedures used to count the MoFR 7-day monitor charts at Bowser and the daily slides used at WFP.

1. DNA Paternity Analyses

Estimates of contamination levels using paternity results were improved by identifying unique genotypes in the surrounding stands at WFP and Bowser. 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 represents the contaminate population, then the ratios of unique genotypes in the orchard to surrounding stands is a more accurate estimate of contamination.

2. Improved Pollen Monitoring Technique

Training sessions were completed for Western Forest Products (Saanich peninsula), MoFR, Puckle Road and Bowser. Follow up training sessions were also completed for WFP and MoFR, Puckle Road. These sessions prove to be very successful. Orchard staff was attempting to identify and count conifer pollen grains with a dissecting microscope at x40 magnification. Neither the type of microscope nor the power of magnification was sufficient to properly distinguish fine pollen grain detail. Since western hemlock and grand fir shed pollen coincidentally with Douglas-fir, it was very difficult to distinguish between these species. Consequently the levels of pollen, especially regionally was substantially inflated.

After training in the use of a compound (transmission) microscope and demonstration of the features distinguishing co-mingling conifer pollen, orchard staff could readily identify pollen grains. The new counting procedures have vastly improved the errors associated with the annual estimates of contamination in coastal Douglas-fir seed orchards.

Based on this approach, the 2004 estimates of pollen contamination at WFP and Bowser were 7.3% and 23.2%, respectively. Interestingly, these values are in close agreement with those pollen monitoring values calculated for 2005. Table 4 shows the re-calculated data for %contamination based on the 2005 surround stand unique genotypes.

Table 5 shows the levels of %contamination estimated using the improved pollen monitoring technique and DNA paternity analyses modified with the surround stand genotypes

DNA paternity analyses produced lower estimates of contamination at the orchard level (Table 5). At Bowser, we selected 14 clones each with two ramets (total of 28 ramets). Of the 28 ramets, 19 were contaminated and the level of contamination ranged from 0 to 15.6% with a mean level of contamination of 5.8%. At Western Forest Products seed orchard we selected 17 clones each with two ramets each (although two clones were bulk lots (i.e, for the whole clone). Of the total number of ramets analyzed (35), 17 were showed some level of contamination and the range of contamination was from 0-21.2% (Table 5). The mean level of contamination for all clones was 2.9%.

Estimates of pollen contamination using pollen monitoring data at the Western Forests Products site on the Saanich peninsula were lower than the mid-island site at Bowser. Contamination for the whole orchard was 5.5%. The mean level of contamination for selected clones in the Western Forest Products seed orchard was 2.2% (Table 6). For the early, mid and late periods of receptivity, the mean contamination levels were 5.1%, 0.89% and 0.82%, respectively.

Pollen contamination in the mid Island, MoFR Seed Orchard at Bowser for the whole orchard was 21.5% (Table 5). If contamination was calculated for the receptivity period of selected clones (Table 7), the mean level of contamination was 1.5% lower (20.1%). Contamination levels for clones also varied by the receptivity period (early, mid and late), with mean values of 37.6%, 17.2% and 5.5% for each of the early, mid and late receptivity periods, respectively.

C. 2006

Estimates of contamination levels in 2006 were intended to test a second year of the improved pollen monitoring and DNA paternity analysis. The crop at both WFP and MoFR Bowser seed orchards were light. However, the few cones that could be located at Bowser in the spring had mostly aborted by the fall. Consequently only five clones with a few seed cones could be found. Of the cones collected, only 34 seed were available for analysis, too few to make any meaningful inferences.

However, at WFP, two ramets from each of 16 clones were selected and enough seed extracted to analyze about 30 seed per tree. Table 8 shows the estimates of contamination from pollen monitoring and DNA paternity analysis. Estimate of %contamination from pollen monitoring was 12.2% (orchard level) and 18.7% (Table 9) for clones. Estimate of %contamination from DNA paternity analysis was 11.7%. Once again, the comparison of unique genotypes that arose from the surrounding stands was used to extrapolate the actual level of contamination.

The extrapolation calculation for WFP is based on the genotyping work of the surrounding background trees at WFP, 13.7% were unique (14 unique genotypes out of 102). The 2006/07 parental analysis revealed that 15 seeds out of 937 harvested at the WFP orchard could be assigned to the parentage of the unique genotypes. This value needs to be extrapolated upwards to include the total contamination, as seen below

WFP

Let x be the total number of contaminant seed

$$x = 15 \text{ seeds} / 13.7 * 100 = 109 \text{ seeds}$$

$$\text{Total Contamination \% is } 109 \text{ seeds} / 937 \text{ seeds} * 100 = \mathbf{11.7\%}$$

At Bowser the genotyping work of the surrounding background trees, showed 9% to be unique. The calculations for Bowser do not make any sense, as there were so few samples tested, therefore no conclusion could be drawn. However, for the sake of the exercise, the calculation is as follows:

BOWSER

Let x be the total number of contaminant seed

$$x = 5 \text{ seeds} / 9 * 100 = 56 \text{ seeds}$$

$$\text{Total Contamination \% is } 56 \text{ seeds} / 34 \text{ seeds} * 100 = \mathbf{164.7\%}$$

Discussion

Pollen contamination is considered a serious problem in all north temperate seed orchard programs. Di-Giovanni and Kevan (1991) provide a summary of pollen dynamics in seed orchards and they report contamination ranges for several species to be 30 to 90%. In Finland, pollen contamination in Scots pine seed orchards ranged from 35 to 76% (Pakkanen et al. 1991) which was also associated with a reduction in frost hardiness of the progeny (Aho and Pulkinen 1993). In maritime pine, Plomion et al. (2001) used chloroplast microsatellites (a technique similar to that used in this research) to find a minimum of 36% pollen contamination. They further determined that the expected genetic gain of orchard seed would have to be reduced between 28 and 50%.

Coastal Douglas-fir orchards located on sites that include an abundance of natural stands that flower regularly are also at high risk. Contamination levels have been estimated in the range of 42 to 60% (Adams et al. 1997) in an Oregon orchard and 16% (Wheeler and Jech 1986) in a Washington orchard. El-Kassaby and Davidson (1990) were able to reduce the levels of contamination in Saanich, British Columbia Douglas-fir orchard to 0% using a combination of bloom delay (see Fashler and El-Kassaby 1987) and supplemental mass pollination. However, this was only one year's estimate and we know contamination levels will vary by year and certainly by orchard site.

All of the above studies for Douglas-fir used isozyme technique. However, as DNA markers are far more variable (polymorphic) than isozyme markers, we are better able to unambiguously distinguishing contaminant pollen from orchard pollen.

Estimates of pollen contamination using 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 the second year and errors associated with estimates of contamination levels from both pollen monitoring and DNA analysis were identified.

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 more reliable 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. Data collected in 2006 at WFP provided estimates of contamination from pollen monitoring at 12.2% and 11.7% from DNA paternity analysis.

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, modifications made to the pollen monitoring and DNA paternity techniques made estimates of contamination that differed by only a few percent and are well within the errors we can tolerate.

Pollen cloud density values for 2004, 2005 and 2006 were light to moderate, 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 will be more reliable.

Since pollen monitoring will be the method of choice to assess annual levels of contamination, we must now standardize the procedure for all orchards. The results from this study were presented to the Coastal Technical Advisory Committee of the Forest genetics Council in early February, 2007. Action arising from this presentation was the forming of a committee to review the methods for estimating the annual level of contamination and make recommendations for standardizing these procedures for all coastal Douglas-fir seed orchards.

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Table 1: Levels of 2004 Douglas-fir pollen contamination in Western Forests Products, Saanich Seed Orchard (166) and the Ministry of Forests, Bowser Seed Orchard (162) comparing both pollen monitoring and DNA data.

2004 Pollen Contamination in Douglas-fir		
Pollen Monitoring	WFP	Bowser
%Orchard	100%	47%
%Clones	81%	45%
DNA Paternity Analyses		
Number Clones Surveyed	23	15
Number of Clones Contaminated	8	6
Number of Seed Surveyed/Clone	32	32
Range of Contamination	0-8.0%	0-22.2%
Mean Contamination	1.3%	2.1%

Table 2: Levels of 2004 Douglas-fir pollen contamination in the Ministry of Forests, Bowser Seed Orchard (162) comparing estimates from pollen monitoring and DNA data (by phenology period).

Clone	Receptivity Period	Pollen Load		%Contam	%Contam
		Orchard	Region	REG/ORC	DNA
212	Apr 9-13	17.2	11.3	65.6	22.2
169	Apr 9-13	17.2	11.3	65.6	2.8
3456	Apr 9-12	15.6	11.2	71.6	3.1
3448	Apr 10-14	16.7	10.8	64.8	0.0
192	Apr 10-13	16.3	10.8	66.2	2.8
			Mean E	66.8	6.2
3414	Apr 16-20	6.7	1.5	22.7	0.0
327	Apr 13-18	12.1	1.6	13.2	0.0
648	Apr 13-18	12.1	1.6	13.2	2.8
69	Apr 15-20	10.8	1.8	16.9	na
3451	Apr 14-19	11.1	1.8	15.8	0.0
3446	Apr 15-20	10.8	1.8	16.9	na
561	Apr 14-18	10.6	1.5	14.3	0.0
			Mean M	16.1	0.6
120	Apr 15-20	10.8	1.8	16.9	2.8
399	Apr 19-23	1.0	0.5	56.3	0.0
80	Apr 16-19	6.6	1.4	21.7	0.0
129	Apr 19-23	1.0	0.5	56.3	na
80	Apr 20-24	0.4	0.3	72.1	0.0
			Mean L	44.6	0.7

Table 3: Levels of 2004 Douglas-fir pollen contamination in the Western Forest Products, Saanich Seed Orchard (166) comparing estimates from pollen monitoring and DNA data (by phenology period).

Clone	Receptivity Period	Mean Pollen Load		%Contam	%Contam
		Orchard	Region	REG/ORC	DNA
3328	Apr 4-8	1.3	1.3	107.2	na
3328	Apr 4-8	1.3	1.2	98.2	0.0
3338	Apr 2-6	1.3	1.0	78.0	na
3338	Apr 2-7	1.3	0.9	68.6	na
3342	Apr 2-7	1.3	0.9	75.6	0.0
3346	Apr 2-7	1.3	0.9	69.5	0.0
3349	Apr 2-8	1.6	0.8	54.1	0.0
			Mean E	78.7	0.0
38	Apr 4-8	1.3	1.3	107.2	2.9
38	Apr 5-10	1.6	1.2	78.5	0.0
299	Apr 4-8	1.3	1.0	78.0	0.0
299	Apr 6-10	1.3	0.9	65.4	0.0
421	Apr 4-6	0.9	0.9	100.7	0.0
421	Apr 5-10	1.6	0.9	55.6	2.8
508	Apr 4-8	1.3	0.8	67.6	0.0
508	Apr 6-8	0.6	1.1	169.1	8.0
3351	Apr 5-8	0.9	0.9	105.4	5.0
3351	Apr 5-8	0.9	0.8	96.2	3.7
3353	Apr 4-8	1.3	0.8	66.4	0.0
3353	Apr 6-10	1.3	1.1	82.7	0.0
			Mean M	89.4	1.9
489	Apr 6-9	1.3	0.9	65.4	2.9
489	Apr 8-11	1.0	0.6	63.7	na
3226	Apr 8-11	1.0	0.7	70.4	0.0
3226	Apr 9-12	0.9	0.7	81.8	2.8
3351	Apr 9-12	0.9	0.7	83.5	5.0
3351	Apr 9-12	0.9	0.7	83.2	3.7
			Mean L	74.7	2.9

Table 4: Levels of 2004 Douglas-fir pollen contamination in Western Forests Products, Saanich Seed Orchard (166) and the Ministry of Forests, Bowser Seed Orchard (162) re-calculated using the 2005 data for surround genotypes.

DNA Analyses	WFP Saanichton	MoFR Bowser
2004 Seed Crop		
Total Seed	597	526
Non-Orchard Males	6	11
%Contamination	1.30%	2.10%
BackGround Sampled		
Total Trees Sampled	102	100
Unique Genotypes	12	9
%Unique Genotypes	11.80%	9.00%
Extrapolated %Contamination	8.5%	23.2%

Table 5: Levels of 2005 Douglas-fir pollen contamination in Western Forests Products, Saanich Seed Orchard (166) and the Ministry of Forests, Bowser Seed Orchard (162) comparing estimates from the improved pollen monitoring procedures and DNA paternity analysis.

2005 Pollen Monitoring	WFP	MoFR
%Orchard	5.50%	21.50%
%Clones	2.20%	20.10%
DNA Paternity Analyses		
Number Clones Surveyed	17	14
Number of Ramets	35	28
Number Contaminated Ramets	17	19
Range of Contamination	0-21.2%	0-15.6%
Mean %Contamination	2.90%	5.80%

Table 6: Estimates of %contamination by clone based on pollen monitoring data (2005) for each of three phenological periods in the Western Forest Products Orchard (166). Mean data for all regional stations and the station closest to WFP (Saanich peninsula hospital) are shown

Pheno Class	%Contam	
	All	Sann Pen
Early	5.02	5.92
Mid	0.89	1.18
Late	0.82	1.06
Mean	2.25	2.72

Table 7: Estimates of %contamination based on pollen monitoring data (2005) for each of three phenological periods in the MoFR Bowser Seed Orchard (162).

Pheno Class	%Contam
Early	37.6
Mid	17.2
Late	5.5
Mean	20.1

Table 8: Levels of 2006 Douglas-fir pollen contamination in Western Forests Products, Saanich Seed Orchard (166) and the Ministry of Forests, Bowser Seed Orchard (162) comparing estimates from the improved pollen monitoring procedures and DNA paternity analysis.

2006 Pollen Monitoring	WFP	MoFR
%Orchard	12.20%	na
%Clone	18.70%	na
DNA Paternity Analyses		
Number of Clone Surveyed	16	5
Number of Ramets	32	6
Number Contaminated Ramets	10	na
Mean %Contamination	11.70%	na

Table 9: Estimates of %contamination by clone based on pollen monitoring data (2006) for each of three phenological periods in the Western Forest Products Orchard (166).

Pheno	
Class	%Contam
E	32.5
M	19.5
L	6.6