

FSP 2004/05
Annual Technical Report

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

Project Y051110

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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 and one in Sechelt. 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.

The objective of this first year project was to measure the extent and magnitude of Douglas-fir pollen contamination in two contrasting orchard sites on Vancouver Island

using pollen monitoring technique. Calculated levels of pollen contamination were then verified using the more robust DNA paternity analyses.

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 (OL) to regional stations located at a distance from the orchard (RL). The extent of pollen contamination is measured over the duration of receptivity of each orchard clone. The ministry standards 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 is different. Previous experience comparing capture levels of slides versus 7-day monitors clearly shows that the 7-day recorders capture more pollen per 24 hour period than the slide technique. 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 calculated as the mean of daily orchard catch (OL) to daily regional catch (RL). The mean of at least eight days early catch is recommended.

Total orchard contaminate pollen is then calculated by multiplying the mean OAF by the total daily regional pollen catch over the receptivity period of the orchard. The percent pollen contamination is then calculated as the ratio of total contaminate pollen to total orchard pollen (x100%) over the receptivity period of the 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). The proportion of contamination was then determined using the calculation described

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.

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. Four cones from each selected tree were collected in the fall, hand extracted and filled seed per cone determined using x-ray analysis.

Results

The mean level of contamination in the two coastal Douglas-fir seed orchards ranged from a low of 1.3% to a high of 100%. Estimating pollen contamination by pollen monitoring produced the highest estimates while the lowest estimates were from DNA analyses. Table 1 summarizes the levels of contamination calculated from pollen monitoring and determined from DNA paternity analyses.

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).

2004 Pollen Contamination in Douglas-fir		
Pollen Monitoring	WFP	MoF
%Orchard	100%	47%
%Clones	81%	45%
DNA Paternity Analyses		
Number Clones Surveyed	23	15
Number Clones Contaminated	8	6
Range of Contamination	0-8.0%	0-22.2%
Mean Contamination	1.3%	2.4%

Pollen contamination in the mid Island, MoF Seed Orchard at Bowser for the whole orchard was 47.2% (Table 2). Since pre-orchard shed pollen monitoring data was not useful in estimating the orchard adjustment factor (AOF), an OAF factor of 1 was used. For Bowser, this is reasonable since the same monitoring technique (7-day recorders) is used for both orchard and regional pollen catch. Contamination was also calculated for the selected clones. The mean level of contamination for clones was 2% lower (45%). Contamination levels for clones also varied by the receptivity period (early, mid and late) it was selected in. Table 3 shows mean levels of contamination to be 66.8%, 16.1% and 44.6% for each of the early, mid and late receptivity periods, respectively.

DNA paternity analyses showed a completely different result. The DNA methods used to unambiguously distinguish all 34 clones in the MoF Bowser orchard are shown in Table 4. Clones with a different letter in a column are distinguishable. Of the 15 selected clones used for paternity analyses (seed) only five were contaminated (Table 5) and mean level of contamination was 2.4%. Range of contamination for the 5 contaminated clones was 2.8%-22.2%.

The calculated level of pollen contamination in the Western Forest Products seed orchard on the Saanich peninsula using pollen monitoring data for the whole orchard was 121% (Table 6). This of course is not correct and the error was principally attributed to the two distinctly different methods for capturing pollen (slides for orchard monitoring and 7-day recorders for regional monitoring) and the missing data from regional counts to calculate the orchard adjustment factor. The OAF for Western was calculated using late pollen capture data (0.34) but it still did not reduce the adjusted regional total sufficiently to yield meaningful results.

The mean level of contamination for selected clones in the Western Forest Products seed orchard was 81% (Table 1). As for the Bowser seed orchard, contamination levels also varied by receptivity period. For the early, mid and late periods the contamination levels were 78.7%, 89.4% and 74.7%, respectively.

DNA paternity analyses of seed from selected clones in the Western Forest Products seed orchard also showed a completely different result. The DNA methods used to unambiguously distinguish all 38 clones in the orchard are shown in Table 8. Again clones in a column followed by a different letter are distinguishable. Of the 23 selected clones used for paternity analyses (seed) only eight were contaminated (Table 9) and

mean level of contamination was 1.3%. Range of contamination for the 5 contaminated clones was 2.8%-5.0% (Table 9).

The levels of contamination estimated using DNA molecular technique are based on one important assumption that the unique clones identified in Tables 4 and 8 are also rare (i.e., absent) in the natural stands and therefore their pollination success is a true indication of pollen dynamics in the orchard. The non-unique clones may have a similar genetic make-up as some background trees and we may classify seeds with these non-unique genetic markers as orchard pollinations when in fact they also could be outside pollinations. If contaminate pollen were classified as orchard pollen, then we would expect a substantially higher pollination success rate in the non-unique clones compared to the unique parents success rate. By comparing the two rates of success, we can infer if there is pollen influx from the outside that we falsely assign to within-orchard parents. If the two success rates are more or less the same, than we know that there is no influx from the outside on top of the clearly and unambiguously identifiable pollen that could not originate from within the orchard.

For example, in the MoF Bowser orchard, 11 clones were unambiguously identified (see Table 4). These are a total of 355 ramets in the orchard of these unique clones. From the 530 seed tested from the 15 selected clones, 174 seed were sired by these 11 unique clones. Each ramet then sired, on average $174/355 = 0.4901$ seeds. This is based on the assumption that these unique clones are also unique in the background pollen donors. This information is not known but for this exercise, the assumption is made if the unique and non-unique clones are similar, then our analysis should show that the non-unique clones sired, on average, more than 0.4901 seeds/ramet. To check if the non-unique clones sired as many seeds as the unique ones, the number of unique ramets (355) is subtracted from the total number of ramets in the orchard (1051) equaling 696. Furthermore, if the total number of seed sired by the unique clones (174) is subtracted from the total number of seed analyzed (530), the result is 356 seeds sired by non-unique clones. The average male success ($356\text{seed}/696\text{ramets}$) is 0.51. Since the ratio of sired seed/ramets for unique and non-unique clones are similar (i.e., 0.49 and 0.51), we can conclude that the pollination success of unique and non-unique clones is the same and the only contamination is observed in the seed whose male parent does not exist in the orchard. Thus, only 11 seeds in total were sired by outside pollen out of the 530 evaluated or a total of 2% contamination.

A similar exercise can be used for the Western Forest Products seed orchard. Five clones are unique, having a total of 31 ramets in the orchard. These 31 ramets sired collectively 71 seeds (of a total sample of 591 seed). Thus, each unique ramet sired ($71/31$) 2.3 seeds/ramet. The remaining ramets ($301-31=270$) sired ($591-71=520$ seeds). Thus each non-unique ramet sired $520/270=1.9$ seeds/ramet. Since the ratios are very similar, we can conclude there is no significant influx from the outside pollen sources. Therefore, the 5 seeds, whose parentage is unknown, are the contaminants for a total of 0.8% contamination.

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 what will be used in this proposal) 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.

In the present study, estimates of pollen contamination using pollen monitoring and molecular techniques produced very different results. Pollen monitoring data collected for one year suggest that the current protocol (pollen monitoring) for estimating pollen contamination in coastal Douglas-fir seed orchards is over estimating the levels of contamination. There may be several reasons for this. On the Saanich peninsula, the regional monitors were put out too late in 2004 to provide any meaningful pollen capture data for the orchard managers to calculate the orchard adjustment factor. This was a problem of communication and can be easily rectified. Furthermore, the pollen capture efficiencies of the two monitoring techniques (slides versus 7-day recorders) are vastly different making them very dependent on the calculation of the orchard adjustment factor. It was also noted that the height of the slide recorders (about 2 m) in the Western Forest Products orchard was in the mid to lower crown region. Positioning the monitors higher in the crown would likely result in higher pollen capture counts. Finally, the procedures for counting the slides and expressing the counts on an area basis may be improved by a different sampling procedure. All of these concerns will be addressed as specific objectives in the second year proposal.

Pollen monitoring technique at Bowser does not have the same problem of capture efficiencies since the same 7-day recorders are used for both orchard and regional monitoring. However, two of the four monitoring sites (Cook Creek and the Met Station) are located within a young stand of trees making the issue of sampling pollen in a stream of air more problematic.

A final comment about the pollen monitoring procedure used in the coastal Douglas-fir seed orchards is how the charts/slides are counted. All orchards use dissecting microscopes with a magnification of x40. Douglas-fir pollen is among the largest of conifer pollen (80-90 microns) and is relatively easy to identify at x40. However, western hemlock pollen is also about the same size (70-80 microns) and sheds about the same time as Douglas-fir. Western hemlock pollen can be readily identified (at x100 with a transmission microscope) from Douglas-fir (sculpturing on the excine) but at x40, this may result in more errors.

DNA paternity analysis avoids all of the problems associated with pollen monitoring. However, the laboratory procedures are very technical, expensive and may not lend themselves to routine analyses of bulk seedlots. As a tool to verify pollen monitoring procedures, DNA analysis is very useful. We are also concerned that inferences from bands on a gel arising from unique and non-unique clones only come from orchard clones may not be sound. We need to determine the extent of these bands in the surrounding natural stands. This will give a higher degree of confidence when gels are scored in paternity analyses. This also is a specific objective for the second year proposal.

Finally, observations for one year are not sufficient to make specific recommendations. We do not know if these results will be similar in another year where either crop intensity or contaminate pollen load is different.

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. The range of estimates for pollen contamination (1-100%) is not acceptable. While there is some uncertainty that the results of the DNA analysis are low, we do not expect these values to rise substantially, even with the knowledge of the extent of unique and non-unique banding patterns in the natural population.

It is very important that we develop a reliable estimate of pollen contamination. Since these contamination values must be included in the yearly calculation of the seedlots genetic worth, errors as high as those calculated for pollen contamination from pollen monitoring could substantially reduce the genetic worth of the seedlot and thereby reduce its commercial value. 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.

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Table 2: Pollen monitoring data (2004) used to calculate orchard levels of pollen contamination in the MoF Bowser Seed Orchard (162). The highlighted colour represents the receptivity period of the orchard.

Date	Mean Pollen Catch g/mm2/d		
	Mean Orch 1-3	Mean Regional	
Mar 30	0.00	0.00	Orchard Pollen Load (=Sum Apr06-Apr20)=30.48 Regional Pollen Load (=Sum Apr06-Apr20)=14.38
Mar 31	0.00	0.00	
Apr 01	0.00	0.00	%Contamination (RL/OL)*100=47.2% OAF=1
Apr 02	0.00	0.00	
Apr 03	0.03	0.00	
Apr 04	0.03	0.00	
Apr 05	0.03	0.00	
Apr 06	1.40	0.91	
Apr 07	0.26	0.12	
Apr 08	0.45	0.25	
Apr 09	0.90	0.49	
Apr 10	1.31	0.63	
Apr 11	6.90	6.88	
Apr 12	6.49	3.19	
Apr 13	1.57	0.09	
Apr 14	0.38	0.01	
Apr 15	4.10	0.31	
Apr 16	2.06	0.23	
Apr 17	3.13	0.78	
Apr 18	0.91	0.18	
Apr 19	0.55	0.24	
Apr 20	0.06	0.08	
Apr 21	0.05	0.00	
Apr 22	0.26	0.21	
Apr 23	0.03	0.00	
Apr 24	0.01	0.00	
Apr 25	0.00	0.00	
Apr 26	0.02	0.00	
Apr 27	0.51	0.15	
Apr 28	0.00	0.01	
Apr 29	0.00	0.00	
Apr 30	0.00	0.00	
May 01	0.05	0.02	
May 02	0.00	0.00	
May 03	0.00	0.00	
May 04	0.00	0.00	

Table 3: Pollen monitoring data (2004) from Table 2 used to calculate clone level of pollen contamination for each of three phenological periods in the MoF Bowser Seed Orchard (162).

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

Table 4: DNA genotyping methods used to unambiguously identify clones for the MoF Bowser Seed Orchard (162) using polymerase chain reaction (PCR), restriction enzymes polymorphisms or sequencing where necessary. Clones within a column with a different letter are distinguishable.

Clone	PCR	Restriction	Sequencing
192	a		
243	b		
399	c		
508	d		
561	e		
641	f		
3446	g		
3453	h		
3455	i		
3456	j		
3577	k		
69	l	a	
80	l	b	a
113	l	b	b
327	l	b	c
129	m	a	
648	m	b	
212	m	c	a
421	m	c	b
3585	m	c	c
3381	n	a	
3451	n	b	
242	o	a	a
3454	o	a	b
401	p	a	a
143	p	a	b
169	r	a	a
299	r	a	b
120	r	a	c
543	r	a	d
593	r	a	e
3414	r	a	f
3432	r	a	g
3448	r	a	h

Table 5: Summary of the clones selected (2004) from the MoF Bowser Seed Orchard (162) for DNA analyses, their phenology period and the number of seed per sample attributed to orchard and non-orchard sources.

Clone	Pheno Period	Total Seed	Orchard Male	Non-Orchard Male	%Contamination
3456	E	32	31	1	3.1
3448	E	36	36	0	0.0
212	E	36	28	8	22.2
169	E	36	35	1	2.8
192	E	36	35	1	2.8
561	M	36	36	0	0.0
3451	M	36	36	0	0.0
327	M	36	36	0	0.0
648	M	36	34	1	2.8
3451	M	36	36	0	0.0
3414	M	34	34	0	0.0
80	L	32	32	0	0.0
80	L	36	36	0	0.0
120	L	36	35	1	2.8
399	L	36	36	0	0.0

Table 6: Pollen monitoring data (2004) used to calculate orchard levels of pollen contamination in the Western Forest Products Seed Orchard (166). The highlighted colour represents the receptivity period of the orchard.

2004	Mean Daily Pollen Catch g/mm ² /d			
	Orch 166	Regional	Saan Hosp	
Date				
Mar-17	0.00	n/a	n/a	Orchard Pollen Load (=Sum Apr01-Apr13)=2.69 Regional Pollen Load (=Sum Apr01-Apr13)=9.56
Mar-18	0.00	n/a	n/a	
Mar-19	0.00	n/a	n/a	Adjusted Regional Load (RL)OAF=(9.56)(0.34)=3.25
Mar-20	0.00	n/a	n/a	
Mar-21	0.00	n/a	n/a	
Mar-22	0.00	n/a	n/a	%Contamination (Adjusted RL)/(OL)100=(3.25)/(2.69)100=120.9
Mar-23	0.00	n/a	n/a	
Mar-24	0.56	n/a	n/a	
Mar-25	0.13	n/a	n/a	
Mar-26	0.00	n/a	n/a	
Mar-27	1.38	n/a	n/a	
Mar-28	0.56	n/a	n/a	
Mar-29	3.31	n/a	n/a	
Mar-30	3.25	1.87	1.05	
Mar-31	0.25	0.48	0.31	
Apr-01	0.25	0.74	0.68	
Apr-02	0.13	1.20	0.53	
Apr-03	0.19	1.12	0.67	
Apr-04	0.38	0.74	0.25	
Apr-05	0.25	1.09	0.44	
Apr-06	0.31	0.99	0.81	
Apr-07	0.00	0.45	0.34	
Apr-08	0.31	0.67	0.85	
Apr-09	0.69	0.40	0.48	
Apr-10	0.00	0.34	0.23	
Apr-11	0.00	0.65	0.21	
Apr-12	0.19	0.71	0.85	
Apr-13	0.00	0.45	0.56	
Apr-14	0.01	0.33	0.36	0.03
Apr-15	0.25	0.97	0.90	0.26
Apr-16	0.01	0.26	0.19	0.04
Apr-17	1.00	0.47	0.36	2.14
Apr-18	0.01	0.42	0.55	0.02
Apr-19	0.31	1.08	1.34	0.29
Apr-20	0.13	0.80	0.67	0.16
Apr-21	0.06	0.37	0.19	0.17
Apr-22	0.06	0.37	0.47	0.17
Apr-23	0.06	0.67	0.46	0.09
	1.91	5.73	OAF	0.34

Table 7: Pollen monitoring data (2004) from Table 6 used to calculate clone level of pollen contamination for each of three phenological periods in the Western Forest Products Orchard (166).

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

Table 8: DNA genotyping methods used to unambiguously identify clones for the Western Forest Products Seed Orchard (166) using polymerase chain reaction (PCR) or, restriction enzymes polymorphisms. Clones within a column with a different letter are distinguishable.

Clone	PCR	Restriction
3342	a	
561	b	
3353	c	
3356	d	
243	e	
3322	f	
3324	g	
38	g	
3328	h	a
3349	h	b
3347	i	a
508	i	
3345	i	
<u>101</u>	j	a
3357	j	
3346	j	
3355	j	
3344	l	
299	l	
3331	l	
3333	l	
3334	l	
3352	l	
3360	l	
3338	l	
421	m	a
241	m	b
489	m	c
226	m	
3330	m	
3447	m	
3362	m	
3450	n	
34	n	
3226	o	
625	o	
449	p	
3351	p	

Table 9: Summary of the clones selected (2004) from Western Forest Products Seed Orchard (166) for DNA analyses, their phenology period and the number of seed per sample attributed to orchard and non-orchard sources.

Clone	Pheno Period	Total Seed	Orchard Male	Non-Orchard Male	%Contamination
421	E	31	31	0	0.0
421	E	35	34	1	2.9
3346	E	16	16	0	0.0
3349	E	8	8	0	0.0
3342	E	27	27	0	0.0
3328	E	36	36	0	0.0
3349	E	12	12	0	0.0
299	M	16	16	0	0.0
299	M	36	36	0	0.0
3353	M	34	34	0	0.0
38	M	16	16	0	0.0
38	M	35	34	1	2.9
421	M	8	8	0	0.0
421	M	35	35	0	0.0
508	M	36	35	1	2.8
508	M	4	4	0	0.0
3353	M	25	23	2	8.0
3351	M	2	2	0	0.0
3351	M	20	19	1	5.0
489	L	27	26	1	3.7
3226	L	35	34	1	2.9
3226	L	35	35	0	0.0
489	L	36	35	1	2.8