Estimating Pollen Yield From Western White Pine: Preliminary Studies

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Two preliminary studies were conducted to develop a method for quantifying the number of pollen cones and pollen yield by western white pine (Pinus monticola *D. Don). In study 1, pollen-bearing shoots from 10 trees in a single stand were sampled to obtain shoot attributes and determine pollen cones per shoot. "Pollen zone" (PZ), the length of shoot occupied by pollen cones, was the most useful feature to predict the number of pollen cones per shoot (CONES). The regression of pollen cones on PZ differed among trees, indicating the need for separate data per clone for very accurate estimates. In study 2, pollen volume per cone collected from two more trees averaged 0.22 cm^3; an average shoot bore 22.4 cones and produced 4.9 cm^3 of pollen. Procedures for estimating the pollen crop and for collecting a specified volume are offered.* Tree Planters' Notes 46(2):64-69; 1995.

Tree-improvement programs for western white pine *(Pinus monticola* D. Don) exist throughout its range. Seed orchards have been established, or are planned, in all programs to produce regular crops of high-quality seeds for reforestation. Like other pines, western white pine is slower to produce pollen cones than seed cones, prompting most orchard managers to collect and apply pollen to promote greater cone retention and increase seed production. Such supplemental pollination allows managers to broaden the seed-crop gene pool and can offset imbalance in reproductive output among orchard genotypes (Schoen and others 1986).

Despite the widespread focus on pines in genetic and tree-improvement programs, little information was found concerning the number of pollen cones per shoot. Himalayan pine (*P. griffithii* McClelland), a "soft" pine like western white pine, has 15 to 35 pollen cones per shoot (Konar and Ramchandani 1958), whereas Chir pine (*P. roxburghii* Sarg.), a "hard" pine, has 120 to 140 (Konar 1960).

Yield of pollen per cone in pines has been reported seldom. Ho and Owens (1973) reported an average of 15 pollen cones per shoot, yielding an average of 8.9 million grains, on three trees of lodgepole pine *(P. contorta* Loud.). Jett and others (1993) estimated pollen yield by loblolly pine (*P. taeda* L.) as 6.2% of the volume of cones, and of 4.5% for Scots pine *(P. sylvestris* L.). The only comparison of pollen yield and cone mass found was that made by Sarvas (1962), who found that the weight of Norway spruce *(Picea abies* (L.) Karst.) pollen produced was approximately double the weight of exhausted pollen cones. Caron and Powell (1989) found a positive correlation between the number of trapped pollen grains and the number of pollen cones per tree.

Hoff and Coffen (1982) recommend collecting data yearly on the seed-cone and pollen crops in western white pine seed orchards in order to quantify the balance in reproductive effort. They represented the pollen crop in classes of catkin (that is, pollen-cone) clusters, rather than as numbers of cones or volume of pollen.

Because no data were found expressing pollen yield per shoot in western white pine, two small studies were conducted. Study 1 was designed to test for the relationship between shoot and pollen-zone lengths and number of pollen cones per shoot, whereas study 2 focused on the relationship between the number of pollen cones per shoot and the amount of collectable pollen per pollen cone ("catkin" or microstrobilus). The results are intended to assist orchard managers in estimating pollen crops more accurately and tree breeders and orchardists in collecting desired amounts of pollen.

Materials and Methods

Study 1. *Estimating pollen buds per shoot.* Ten open-grown trees in a natural stand of western white pine in British Columbia described in El-Kassaby and others (1987) were selected during a "good flowering year." Trees ranged in age from 34 to 38 years, from 6 to 10 m in height and from 12 to 25 cm in dbh. Prior to sampling, each tree was assessed visually for vertical distribution of pollen-bearing branches and for variation in the length of pollen shoots. Samples were taken prior to shedding when pollen buds were clearly visible. Trees were climbed and 19 to 21 pollen-bearing shoots (figure 1) per tree were severed individually from branches located throughout the crown; however,

Figure 1— *Pollen-bearing shoot of western white pine* (Pinus monticola *D. Don*).

no procedure was established to sample all shoot lengths equally. All shoots from a tree were placed in the same bag and stored in a refrigerator (2 ± 1 ºC) for no more than a month until examined. By tree, the following data were recorded for each shoot: shoot length (SL) (shoot base to base of terminal bud) in centimeters, pollen zone (PZ) (length of shoot occupied by pollen cones) in centimeters, and number of pollen cones (CONES). Analyses of variance (ANOVA) to test for differences among trees were conducted on raw data or on percentage data with and without arcsin transformation of the square root of percentage value per shoot (Zar 1984), and on CONES data following covariance removal of differences in SL, PZ, and both factors combined. Regressions to predict CONES were calculated from both SL and PZ, and for the percentage of the total shoot length that has pollen cones on it ("POL %" = 100% [PZ] ÷ SL), separately and combined, by tree and for all trees pooled. Regressions from separate trees were compared by covariance for the significance of differences in slope and intercept, using SAS GLM procedures and *t*-tests (SAS 1989).

Estimating sample size per tree. Sample sizes to determine mean PZ and mean CONES were calculated by tree using the following formula (Zar 1984):

$$n = (t_a^2 \; C \; SD^2 \bullet F_b) \div D^2$$

where "t_a" = the tabular "t" = value for degrees of freedom "a" and type 1 error probability of 0.05, "SD" = the standard deviation of the parameter (for example, mean PZ), "F_b," = the probability of committing a type 2 error, set here at 0.10, and "D" = half of the acceptable limit sought. "D" was set at 0.5 cm for PZ and at approximately 25% of the mean of CONES by rounding up to the nearest full number.

Study *2. Estimating pollen quantity per bud.* Twenty pollen-bearing shoots per tree were collected by the same method from two more trees in the stand sampled for study 1 at the commencement of pollen shedding by each shoot. Each shoot was placed in a separate paper bag in the field, then placed in a warm environment indoors later the same day to continue pollen shedding. Each shoot was re-cut and the base placed immediately into tap water in a small vial in each bag. Water level was checked and replenished daily, as needed, during the study. Pollen was allowed to shed into the bag until tapping the shoot produced no more pollen. All pollen was sieved to remove impurities and poured into a graduated 10-ml flask. After the flask was tapped to loosen pollen adhering to the glass and to level the pollen surface, the volume was read to the nearest 0.5 ml. Finally, CONES per shoot was recorded. Linear regressions to predict pollen volume from CONES were calculated for each parent tree and for all data combined using SAS REG procedures (SAS 1989).

Results

Study 1. Summarized data of shoot attributes appear by tree in table 1. Trees are numbered in order of decreasing mean pollen cones per shoot. The following ranges were recorded for each attribute: SL, 1.6 to 14.8 cm; PZ, 0.7 to 5.8 cm; CONES, 5 to 60; and POL %, 17 to 85%. ANOVA found "tree" to be significant (P #0.05) for each parameter. Mean SL (trees 6 and 5) and mean PZ (trees 6 and 2) differed (P # 0.0001) by a factor greater than 2 (table 1). The lowest mean of POL % found was from tree 5, due mainly to its long shoots, whereas the highest value came from tree 2, due mainly to its high PZ (table 1).

Maximal and minimal values of pollen cones per shoot sampled were 60 buds from tree 2 and 5 buds from tree 10. The lowest mean of pollen cones per centimeter (CONES/cm) came from the tree with the longest shoots (number 5), but with a number of pollen cones per shoot near the mean. The most pollen cones per centimeter were found on tree 1, which had the highest mean value of pollen cones per shoot and a shoot length slightly below average.

Parent trees differed strongly (P # 0.0001) in CONES/cm PZ (table 1). The correlation between CONES and CONES/cm PZ was weak (R = 0.182) and not statistically significant.

Mean pollen buds per shoot before and after removal of SL and PZ effects by covariance, both separately and combined, are presented in table 2. Although all means were affected, the major difference following adjustment of the means is that tree 5, rather than tree 10, displayed the lowest adjusted value. It was consistently low following each adjustment, particularly when the effect of PZ was removed. Conversely, tree 1 ranked highest in all attributes but CONES/cm PZ, where it differed from tree 6 (table 1).

Linear regressions of CONES on shoot attributes were calculated. Greatest agreement (highest R^2) was found with PZ: all trees studied produced regressions significant at 0.0001 (table 3). Much-poorer trends were found with SL (only 5 of 10 regressions significant) and with POL % (3 of 10 significant) (results not shown). Both slopes and intercepts of CONES/cm PZ varied

Table *1-Descriptive of pollen-bearing shoots from 10 western white pine trees.*

	Shoot Length			Pollen zone(PZ)			% Pollen bearing per shoot (POL%)			Pollen Cones shoot (CONES)			CONES/cm			
Tree	Mean (cm)	SNK*	SD	Mean (cm)	SNK	SD	Mean	SNK	SD	Mean	SNK	SD	SL Mean	SNK	PZ Mean	SNK
1	4.31	b	1.66	2.49	a-c	0.88	58.8	ab	10.4	30.6	a	11.0	7.3	a	12.4	b
2	4.97	b	1.97	3.10	a	1.20	63.5	a	8.7	27.3	ab	11.4	5.7	bc	8.9	de
3	4.58	b	1.51	2.25	b-d	0.45	53.1	bc	14.6	23.4	bc	6.2	5.5	bc	10.3	cd
4	6.42	a	3.32	2.64	a-c	0.74	44.7	cd	9.6	22.2	bc	6.5	3.8	de	8.5	de
5	7.23	a	3.04	2.84	ab	0.75	42.0	d	9.9	20.9	b-d	6.6	3.1	a	7.4	e
6	3.05	b	0.73	1.47	e	0.39	48.9	b-d	8.9	20.7	b-d	7.0	6.9	ab	14.0	a
7	4.73	b	2.56	2.09	c-e	1.00	45.7	cd	9.3	20.3	cd	7.7	4.8	cd	10.5	cd
8	4.17	b	1.36	2.20	b-d	0.59	54.2	bc	7.8	18.9	cd	4.6	4.8	cd	8.8	de
9	3.35	b	1.48	1.58	a	0.71	48.9	b-d	12.4	16.6	cd	5.7	5.7	bc	11.5	bc
10	3.44	b	0.78	1.64	de	0.53	49.4	b-d	17.6	14.7	d	6.5	4.5	cd	8.9	de
Mean	4.62		1.84	2.23		0.72	50.9		10.9	21.6		7.3	5.2		10.1	
CV%	39.8			32.5			21.4			33.9			34.1		24.0	

* Means followed by the same letter(s) do not differ statistically (P>0.05) per Student-Newman-Keuls (SNK) test.

SD= standard deviation

Table 2— *Mean pollen cones per shoot before rind after covariance adjustment for shoot length polled-zone length and bout combined*

				Cones/shoot adjusted for:				
Tree	Cones /shoot	SNK*	SL length	*t* group†	PZ length	*t* group†	SL and PZ length	*t* group†
1	30.6	a	31.1	a	28.5	a	27.6	a
2	27.3	ab	26.7	b	20.3	c-a	18.7	d
3	23.4	be	23.5	b-d	23.3	b	23.2	b
4	22.2	be	19.0	a	19.0	d-g	20.0	cd
5	20.9	b-d	16.2	a	16.0	h	17.6	d
6	20.7	b-d	23.5	bc	26.8	a	26.7	a
7	20.3	cd	20.1	cd	21.5	b-d	22.0	bc
8	18.9	cd	19.7	c-a	19.2	c-g	18.7	d
9	16.6	cd	18.9	a	21.9	be	21.9	be
10	14.7	d	16.8	a 1	9.5	c-f	19.4	cd
Mean	21.6		21.6		21.6		21.6	
SD	8.7		6.8		4.5		4.3	
CV%								

* Means followed by the same letters) do not differ statistically (P > 0.05) according to the Student-Newman-Keuls (SNK) test.
† Means followed by the same letter(s) do not differ statistically (P > 0.05) for all-possible t-test comparisons among means.

Table 3– *Regression* of *pollen-cone number on pollen-zone length for ten western white pine trees*

Tree no.	No. of shoots	Slope Intercept (cones)	(cones/cm of PZ)	R‡*	Est. cones/ 2 cm PZ length,
1	20	4.34	10.54	0.727	25.4
2	21	1.00	8.48	0.786	18.0
3	20	-2.66	11.58	0.725	20.5
4	21	3.61	7.03	0.624	17.7
5	19	0.84	7.05	0.652	14.9
5	20	-1.28	14.93	0.688	28.6
7	20	8.58‡	5.63	0.534	19.8
8	20	5.24	6.22	0.627	17.7
9	20	6.58§	6.37	0.622	19.3
10	21	-2.19	10.29	0.715	18.4
Pooled	202	5.33	7.28	0.582	19.9

*Regressions all significant at P= 0.0002 or less.
†Estimated number of pollen buds on 2-cm pollen-zone length using table 3 regression by tree number. Pollen-zone length mean approximately 2 cm in table 1
‡ Tern significant at or below P= 0.01
§ Term significant at or below P=0.005

widely among trees. Comparisons of these regressions by tree showed that fifteen of 45 comparisons (not shown) differed significantly in slope and 24 of the 30 remaining comparisons differed in intercept. Analysis of data from all 10 trees pooled produced the following regression equation (regression eq. #1):

1. Pollen buds per shoot = 5.33 + 7.28 PZ (cm)
$R^2 = 0.58$, P = 0.001.

Estimated number of pollen buds on a 2-cm PZ (approximately the mean BL in table 1), using the regression of pollen buds per cm of PZ by tree, appear by tree in table 3.

Combining SL and PZ as predictors produced a highly-significant (P = 0.01) regression for each tree (not shown), and increased mean R^2 from 0.670 to 0.732, an average of 8.1%G (maximum 27.5% [tree 9], minimum 0% [tree 3]).

Estimating sample sizes per tree. Minimal sample sizes calculated for CONES are smaller than for PZ (table 4). The range of estimated sample size per tree is more than two for CONES, whereas it is greater than five for PZ (table 4).

Study 2. T-tests indicated that the two trees sampled were very similar (P > 0.05) in both pollen cones and volume shed per shoot (table 5). An average shoot bore about 22 pollen cones and produced nearly 5 cm^3 of dry pollen. Comparison of their trends of pollen cm^3 per cone confirmed the similarity of the trees: neither trend had an intercept differing from zero, nor did their slopes differ (P# 0.64). Thus, the

Table 4— *Minimal sample sizes (shoots) require/d to attain specified limits for PZ length and pollen cones per shoot* by *tree*

Tree no.	Mean PZ length (cm)	No. of pollen-bearing shoots	Mean pollen cones/ shoot	No. of pollen-bearing shoots
1	2.49	31	30.6	21
2	3.10	53	27.3	28
3	2.25	11	23.4	14
4	2.64	24	22.2	14
5	2.84	24	20.9	20
6	1.47	9	20.7	22
7	2.09	39	20.3	25
8	2.20	17	18.9	12
9	1.58	22	16.6	16
10	1.64	14	14.7	27
Mean	2.28	24.4	21.6	19.9
SD	0.31	13.6	8.7	5.7

Note: PZ length = estimated within 0.5 cm of mean by tree: pollen cones/shoot =within 25% of mean number of pollen cones by tree.

following pooled regression equation (regression eq. #2)

2.Pollen-shed volume (cm^3) = 0.22 CONES; n=40
R^2 = 0.73, P = 0.001,

will estimate pollen yield per shoot from these trees accurately.

Discussion

Mean pollen cones per shoot from the western white pine trees in this study fall within the values for Himalayan pine *(P. griffithii*) (Konar and Ramchandani 1958) but well below those reported for Chir pine by Konar (1960).

Although no estimates of pollen yield per tree were made, factors influencing the yield per shoot are apparent in tables 1 and 2: trees differed in all shoot attributes, particularly PZ, CONES, and CONES/cm PZ. The importance of PZ on CONES is apparent in table 2 and in results from tree 6 in table 3. 1t should produce nearly twice as many pollen cones as tree 5 (28.6 vs. 14.9) from a 2-cm PZ (table 3), yet this difference in potential fecundity is reduced by the 2-fold difference between the trees in PZ (1.47 cm vs 2.84 cm), resulting in near-equal values: 20.7 pollen cones for tree 6 vs. 20.9 pollen buds for tree 5 (table 1). Thus, PZ is the most useful of these two variables to predict number of pollen cones on a shoot, and little is gained by measuring SL. The relative importance of CONES can be seen in table 2: the adjusted values in column 8 (adjusted for both SL and PZ) generally agree with those in column 6 (adjusted for only PZ). The weak correlation (R = 0.18) between pollen-cone number and pollen cones per centimeter of PZ suggests that differential elongation of shoots carrying a similar number of pollen cones might have occurred. Further study could produce more accurate estimates of cones from PZ, since the latter is the best predictor of pollen cones per shoot. Because the number of pollen cones per shoot for the two trees sampled for determination of cubic centimeters of pollen shed per cone are well within the bounds of confidence obtained for the 10 trees in study 1 (table 1), regression 2 could be applicable to them also, and perhaps to other western white pines, if shoots are collected at the appropriate time. However, Owens and Molder (1977) noted that "... the numbers of pollen cones varied considerably depending upon the year, the tree and the position of the branch on the tree." Sampling more trees and years will test the values presented here. **In the meantime a "rule of thumb" from this preliminary study is that 5 pollen cones will produce 1 cm^3 of pollen.**

The differences in regression of CONES on PZ among trees in study 1 indicate that inter-tree differences in allocation to reproduction per shoot may exist in white pine and may reflect genetic control, as found for loblolly pine by Schmidtling (1983). If so, white pine trees might differ in reproductive output and reproductive success, and perhaps in contribution to the seed crop (Roberds and others 1991). This will have an impact on the genetic balance in seed-orchard seed crops. Thus, depending on the accuracy wanted in estimates of pollen crop or in pollen volume to be derived, a separate regression to estimate CONES or

Table 5— *Summary of pollen cones per shoot and pollen-shed volume (PSV) per shoot for two western white pine trees*

Tree	Pollen cones/shoot				PSV/shoot (cm^3)				Mean cm^3
No.	Min.	Max.	Mean	SD	Min	Max.	Mean	SD	pollen /bud
A	11	44	23.3	9.80	2.0	9.5	5.3	2.15	0.23
B	12	38	21.4	8.22	2.0	8.0	4.6	2.02	0.21
Mean			22.4	8.97			4.9	2.09	0.22
CV%			40.0				42.6		

cubic centimeters of pollen per cone may be needed for each seed-orchard clone, or a different sample size will be required to obtain a specified volume of pollen from each targeted tree or clone.

Applications

Estimating pollen crop per tree:

1. Determining or estimating the number of pollen cones per shoot (CONES): Collect approximately 30 pollen-bearing shoots from throughout the pollenbearing portion of a tree and determine mean and standard deviation of length of pollen zone (PZ) and CONES; calculate the regression of CONES on PZ (SAS 1989):

$$CONES = A + B\ (PZ) \qquad \text{REG 1}$$

2. Estimating volume of pollen shed per shoot: Using mean CONES [or the predicted value of CONES calculated from mean PS using REG 1 (step 1)] and regression eq. #2 from this paper, calculate extractable pollen volume per shoot, that is, extractable pollen (in cubic centimeters) = 0.22 (CONES).
3. Estimate the number of pollen-bearing shoots per tree according to Hoff and Coffen (1982).
4. Derive the estimated pollen crop per tree (in cubic centimeters) by multiplying the values from 2 and 3 above.

Collecting a specified volume of pollen per tree:

5. Determine mean PZ or CONES as in 1 above for the specified tree.
6. Estimate the pollen yield per shoot corresponding to mean or predicted CONES per shoot using REG 1 (step 1) and regression eq. #2 in step 2 above.
7. Divide the volume of pollen desired by the estimate of pollen per shoot from step 6 to obtain the minimal number of pollen shoots needed; collect them.
8. Determine the pollen volume per cone for your collections and compare to the mean of 0.22 cm^3 from this paper for your future use.

NB: To "ensure" collection of sufficient pollen 68% of the time, estimate the number of shoots to collect in the following way:

9. Subtract the standard deviation in CONES or PZ for your sample from the mean obtained in step 5. Then use that resulting value in step 6 and proceed through step 7, using a value of 0.125 cm^3 pollen per bud (derived as mean cm^3 pollen/shoot - SD cm^3 pollen/shoot [4.9 cm^3 - 2.1 cm^3 pollen/shoot] = 22.4 buds/shoot) (table 5).

NBB: To "guarantee" collection of sufficient pollen 95% of the time, use the mean of PZ minus 2 times standard deviation through steps 6 and 7. This should produce considerably more pollen than desired from some trees, permitting g storage of the excess for farther studies or for supplemental pollination in years with smell pollen crops on some clones.

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