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Estimation of gene flow into two seed orchards of loblolly pine (*Pinus taeda* L.)*

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Summary. Multilocus allozyme markers, which were present in background stands but not in two seed orchards, enabled estimation of gene flow into two seed orchards of loblolly pine. Estimates averaged 36% in two orchards over three years. When pollen pool frequencies in the background stands were used rather than gene frequencies in the non-orchard trees, the estimates averaged 60%. In one year, trees in the row on the edge of one orchard received significantly more pollen from background stands. Estimates of gene flow between the two 2-ha orchards average 10%. Implications of the observed levels of gene flow are discussed in terms of population genetics and seed orchard management.

Key words: Allozymes – Gene flow – Loblolly pine – Pollen contamination – Seed orchards

Introduction

Gene flow, the exchange of genetic factors between populations owing to the dispersal of gametes or zygotes (Mayr 1970), is a highly important parameter in plant population genetics. Estimation of gene flow is often difficult; nevertheless, information on gene flow is valuable from both an evolutionary and applied point of view. Recent discussions (Ehrlich 1979) have said that flow is restricted in most organisms. Studies of agricultural plants, both entomophilous and anemophilous, have generally concurred. In anemophilous forest trees, however, pollen has been shown to travel long distances and to maintain its viability over those distances (Lanner 1966). Consequently, the possibility of effective long-distance gene transfer exists in forest trees.

Despite the fact that pollen is capable of movement over long distances, studies based on single trees indicate that most pollen may travel only about 40 m. Based on a diffusion model of pollen dispersion, Strand (1957) argued that the size of the pollen source is very important to the relation between pollen density and distance; and that therefore results obtained from a single tree cannot be applied to a stand. The existence of thermal shells may cause unpredictably long-distant pollen movement which does not conform to existing models (Lanner 1966). The distance travelled by pollen may also depend on factors such as the type of ground cover and the density of the stand (McElwee 1970).

Pollen dispersal of anemophilous forest trees has great practical importance due to the prevalent use of wind-pollinated seed orchards. Seed orchards are plantations of clones or seedlings from selected trees which are cultured for early and abundant production of seed (Snyder 1972). Most genetically improved forest tree seed used in the U.S. is produced in such orchards. These orchards depend upon wind pollination to accomplish pollen transfer among selected parents. This creates the risk of transfer of pollen into the orchard from surrounding unselected trees. If pollen contamination occurs, i.e., if selected parents are pollinated by adjacent trees, a decrease in the genetic gain of trees grown from orchard seed would result. This decrease could be as much as one half, if all the seeds were fertilized by contaminating pollen (Squillace and Long 1982). Dilution strips dividing seed orchards from surrounding stands have been established, but their effectiveness has been questioned (Squillace 1967; Squillace and Long 1982; Jonsson et al. 1976).

In most pollen dispersal studies, amounts of pollen at varying distances from a pollen source are measured. These measures cannot differentiate among pollen sources nor estimate the effectiveness of different sources in fertilizing ovules. The recent use of bio-

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chemical markers has enabled geneticists to estimate the proportion of ovules fertilized by different pollen sources. In this study, allozymes were used to estimate gene flow between two seed orchards, and from the outside stands into the seed orchards.

Materials and methods

The two seed orchards studied were owned by Champion International and located near Newberry, South Carolina. They are composed of grafted ramets of fifty loblolly pine clones. Ramets in the orchards were collected from phenotypically superior ortets located within 110 km of the site. The clones were placed in either the high specific gravity (HSG) or low specific gravity (LSG) orchard based on their wood specific gravity. The orchards were each approximately 2 ha (five acres) in size, separated by a 100 m strip, which included a virginia pine (*Pinus virginiana* L.) seed orchard. The three orchard complex was surrounded by a pollen dilution strip which included a cleared area, a slash pine plantation (*Pinus elliotii* Engelm.) and mixed loblolly-hardwood stand from which all flowering loblolly had been removed for 122 m from the edge of the orchards. Beyond this strip, pollen producing loblolly and mixed loblolly-hardwood stands surrounded the orchard complex.

In 1974, there were 301 ramets of 23 clones in the LSG orchard and 300 ramets of 27 clones in the HSG orchard. These were reduced by roguing in the fall of 1974 to 202 and 183, respectively; these were present for the spring 1975 pollination and the fall 1976 cone collection. The grafts averaged 15 years old, were approximately 12 m high, and had been in full pollen and cone production for several years at the time of this study.

In the fall of 1978, seed samples were obtained from 57 loblolly pine trees in the surrounding stands. The trees were distributed throughout the forested area surrounding the orchard, and were sampled in approximate proportion to the quantity of male strobili produced from each group of trees. Trees to the northwest of the seed orchards had been clearcut before 1978; however, enough scattered trees remained to obtain a sample from ten trees. Four loblolly pine trees were found in the slash pine plantation, and were also sampled. Some of these trees were closer than the 122 m pollen dilution strip and had probably been missed due to their rare ocurrence in the slash pine plantation. These trees were from 50 to 100 m from the border of the orchard, but were in a dense stand and produced very little pollen. All trees chosen for sampling were located within approximately 500 m of the seed orchards.

Phenological measurements were taken during the pollination season of 1979. The timing of male and female flowering of ramets of all clones was observed. Literature suggests the order does not change from year to year, although the entire period can be condensed or extended (Jonsson et al. 1976). Also during 1979, it was noted that some trees in the surrounding stands were both earlier and later than the seed orchard trees; i.e., the flowering period of the orchard lay within the span of the flowering period in natural stands.

In the two seed orchards, seed was sampled from a total of 38 ramets of 24 clones in 1975 and 36 ramets of 25 clones in 1976. Approximately one-half the clones were sampled in each year. These samples will be referred to as ramet samples. Random samples from seed orchard crops for 1975, 1976, and 1978 were also used. Seed crop samples were composed of bulked seed from both orchards. The sampled seed were assayed electrophoretically using the methods of Adams and Joly (1980a). The megagametophyte (1N) and embryo (2N) tissues of the seed were analyzed separately, and the genotype of each tissue was determined at seven allozyme loci:

GDH (glutamate dehydrogenase, E.C.1.4.1.2),
LAP1 (leucine aminopeptidase, E.C.3.4.11.1),
PGI2 (phosphoglucose isomerase, E.C.5.3.1.9),
GOT2 (gluconate oxaloacetate transaminase, E.C.2.6.1.1),
6-P6D (6-phosphogluconate dehydrogenase, E.C.1.1.144),
and PGM1 and PGM2 (phosphoglucomutase, E.C.2.7.5.1).

Details of the banding patterns of these allozymes, and analyses of their Mendelian genetics are also found in Adams and Joly (1980a). Since the megagametophyte contains the same haploid genotype as the female gametes, the parental origin of both genes in the embryo could be inferred. In such a manner, the allelic frequencies among the male gametes (pollen pool) forming the embryos in a seed sample could be determined.

Estimates of contamination

The method which was originally used to estimate contamination was the marker group method (Friedman and Adams 1981; Friedman 1982). The sources of pollen which could contribute to the pollen pool of ramets in one orchard were divided into pollen from each ramet itself (i.e., selfing), from other trees in the same orchard, from clones in the adjacent loblolly pine orchard, and from loblolly trees in the background stands. Self-fertilization was found to account for approximately 1–2% of the progeny of the seed orchard clones in (Friedman 1982), so selfing was disregarded in order to formulate a simplified pollen migration model. The frequency (Pp) of any marker allele (a_m) in the pollen pool of a receptor orchard can be described as a function of the frequencies of the marker alleles in various pollen sources, and the proportion of pollen gametes from those sources that make up the pollen pool of the orchard.

Thus, the model is:

$$P_p=(1-M_A-M_B)P_R+M_AP_A+M_BP_B \quad (1)$$

where

P_p = frequency of allele a_m in the pollen pool of the receptor,
P_B = frequency at which allele a_m is produced in the background stands,
P_A = frequency at which allele a_m is produced in the adjacent orchard,
P_R = frequency at which allele a_m is produced in the receptor orchard,
M_A = the proportion of the receptor pollen pool due to pollen gametes from the adjacent orchard,
M_B = the proportion of the receptor pollen pool due to background pollen sources,
and
$1-M_A-M_B$ = the proportion of the receptor pollen pool due to pollen from the receptor orchard.

Two situations were of interest, one in which markers were unique to background stands, equation 1 reducing to:

$$M_B=\frac{P_P}{P_B}. \quad (2)$$

An estimate of M_B is then $x/n\,P_B$ where x is the number of a_m alleles observed in n sampled pollen gametes in the receptor orchard, and, hence, x/n is an estimate of Pp. The large sample variance of M_B is var $(M_B) = M_B(1-M_BP_B)/P_Bn$. Since P_B and P_p must both be estimated, their sampling variances must also be considered in the variance of M_B, such that:

$$\text{var}(M_B) = \frac{1}{(P_B)^2}\,\text{var}(P_p) + (M_B)^2\,\text{var}(P_B) \qquad (3)$$

The second situation occurs when markers are found in one, but not the other orchard, and not in background stands, such that P_B and P_R are equal to zero, equation 1 reducing to:

$$M_A = \frac{P_p}{P_A}, \text{ with var}(M_A) = M_A(1-M_AP_A)/P_An.$$

The assumptions of both these models require that all genotypes contribute pollen equally to the pollen pool, and that the success of alleles in fertilizing ovules is in proportion to the gene frequencies in the sampled outside trees. In other words, the sampled trees must have the same gene frequencies as those actually contributing to gene flow, and the frequencies in the pollen produced by outside trees must be more or less the same as in the outside trees themselves. It is possible that gene flow may occur from more distant stands, which may have different gene frequencies than those sampled.

Only one single locus marker was found in the background stands at a frequency high enough to estimate gene flow and was also not found in either orchard. Two other alleles unique to the background stands (in the pollen pool) were found, but occurred at such low frequency that the sampled 57 standing trees did not contain any of these alleles, and, hence, their frequencies in the background stands could not be estimated. Therefore, it was necessary to use multilocus markers. Their frequencies were substituted into the above formulae. Since multilocus markers were used, an additional assumption was necessary that the frequency of multilocus genotypes in the pollen pool was equal to the product of their single-locus allele frequencies. This assumption was supported by the fact that the loci segregate independently (Adams and Joly 1980b). This assumption was later tested by Smith and Adams (1983) and found to give equal results to generating all possible allelic combinations for each sampled tree.

The multilocus markers used in this study were placed in marker groups based on the locus of the most rare allele of the combination. Each multilocus combination was included in only one marker group. Since these loci segregate independently, each marker group yielded an independent estimate of gene flow. Breaking down the markers into marker groups was done so that independent estimates could be compared. The estimate of M_B for each marker group was then: $M_B = x/n\,P_B$ where x was now the number of multilocus markers observed in n sampled gametes in the receptor orchard. P_B was estimated as the sum of all the expected frequencies of the multilocus markers in the background stands. The sampling variance on P_p remained the same. However, the sampling variance of P_B was increased in size and complexity, because P_B became the sum of several products of gene frequency estimates which themselves had sampling variances. Twenty multilocus combinations were found which did not occur in orchard clones. These were divided into four marker groups. Marker group 1 was composed of seven two-locus combinations which included allele PGM1–2. Marker group 2 was composed of two three locus combinations which included allele GDH-2. Marker 3 was composed of only one combination, GOT-1 and PGI-4. Marker 4 included the unique allele 6PGD-7, four two-locus combinations with 6PGD-3, three two-locus combinations with 6PGD-1 and two three-locus combinations with 6PGD-5.

In only one situation was it possible to use a single marker allele. LAP2–3 was unique to one clone in the HSG orchard, and was not observed in the background stands (but was observed at very low frequency, 0.003, in the pollen pool of the background stands). Therefore, it could be used to estimate gene flow between the HSG and LSG orchards. No other alleles or multilocus combinations could be used, as no other alleles were found in the orchards which were also not found in the surrounding stands.

It is likely that violations of the above assumptions did occur; that differences among trees in the background stands in production of pollen, phenology of pollen dispersal, and gametic selection, could cause unequal success in fertilization among genotypes. But by using several multilocus combinations in each marker group, reduced sensitivity to these factors was achieved. Perhaps the weakest assumptions are 1) that pollen pool frequencies can be estimated from outside trees, and 2) that the outside trees sampled will reflect the gene frequencies in trees contributing to gene flow. If the gene frequencies in the sampled trees are widely different from those in the pollen actually contributing to gene flow, then the estimates of gene flow for each marker group should vary widely.

The marker group method of estimating contamination was extended by Smith and Adams (1983). Generally, in gene flow estimation, estimates are obtained for each marker and then pooled. In the group method, the markers are added into groups, separate estimates for each group obtained, and then the group estimates pooled. In the Smith and Adams (1983) method, all multilocus combinations are added to arrive at one estimate. The Smith and Adams (1983) method does not take into account the sampling variance around background gene frequency estimates in estimating the variance on m. This provides a minimum variance estimate.

Estimates of both background stand, and orchard-to-orchard gene flow were obtained using the latter procedures. The background stand estimate was calculated by counting the multilocus markers which could only have been produced in the background stands. The frequency at which they were observed in the orchard was then divided by the frequency that they were found in background stands. The total contamination estimate was calculated by counting all combinations which could not occur within one of the orchards. The frequency of non-orchard marker counts was then divided by the combined gene frequency estimate of the background stands and the other orchard, each weighted by one half. To obtain the between orchard estimate, the background estimate was subtracted from the total estimate (D. Smith, personal communication). In this manner, orchard-to-orchard gene flow estimates were obtained in each direction. Finally, the frequencies of the background stand multilocus markers observed in the pollen pool of each ramet were examined to determine the spatial distribution of contamination within the orchards. The frequencies of contaminants for each ramet sampled were first transformed using an arcsine square root transform (Snedecor and Cochran 1967). They were then correlated with spatial and phenological variables, which included distance to the nearest edge of the orchard, coded direction to the nearest edge of the orchard, coded direction to the nearest edge, phenology group of the receptor ramet, the column and row of the receptor ramet, and the proportion of crown exposed to the outside.

Results

The counts of multilocus markers obtained using the marker group method were tested for heterogeneity between orchards and years using a chi-square heterogeneity test. Only one of the eight marker-year combinations (group 4, 1975) exhibited significant heterogeneity over orchards. Therefore, counts were pooled over orchards. Counts were found to be homogeneous over years, so data were also pooled over years. Finally, the seed crop samples were added to the data for a total sample size over orchards and years of over 7,600 seed (Table 1).

Table 1. Estimated proportion (M_B) of progeny of the LSG and HSG orchards (all samples combined) fertilized by pollen from background stands based on four multilocus marker groups

Allozyme marker group			Observed pollen gametes			
No.	P_B[a]	No. multilocus combinations	With markers	Total	(M_B (s.e.)	
1	0.014	7	23	7,626	0.21	(0.16)
2	0.004	2	9	7,663	0.29	(0.16)
3	0.005	1	6	7,703	0.17	(0.11)
4	0.062	10	197	7,609	0.42	(0.11)
Pooled over markers[b]					0.28	(0.06)
Sum[c]	0.084	20	235	7,703	0.36	

[a] Expected frequency of markers in pollen produced by background stands
[b] Weighted by variances
[c] Obtained by adding all marker groups

Four pooled estimates of M_B were obtained, one for each marker group. Estimates ranged from 0.17 to 0.42 among the four marker groups; however, due to the large standard errors, these estimates proved to be homogeneous ($x^2 = 2.77$, 3 d.f., $P > 0.05$). The estimates were pooled by weighting by the variances for an overall gene flow estimate of 0.28 from the background stands. If the multilocus counts were simply added, the estimate would have been 0.36. Because sampling variances were placed around each background stand gene frequency used in the multilocus combinations, the variance for one two-locus marker found at a very low frequency in the background stands (marker group 3, 0.005) was the same as the variance for 10 multilocus combinations expected at 0.062 (marker group 4). The inclusion of sampling variances on each allele frequency used in the multilocus combinations, and then weighting by those variances to get a pooled mean, yielded a different estimate (0.28) than that obtained by adding all multilocus markers.

The Smith and Adams (1983) method for estimating contamination produced similar results, with lower variances, for the reasons discussed above. Due to the lower variances, estimates could be obtained separately for each orchard in each of three years, and separately for the seed crop sample in each of two years (Table 2).

In the LSG in 1976 the contaminant estimate (0.48) was significantly higher than that for 1975 and 1978, based on a weighted by variance chi-square hetero-

Table 2. Estimated proportion (M_B) of progeny fertilized by pollen from background stands based on method of Smith and Adams (1983)

Orchard	Year	n	P_P[a]	P_B[b]	M_B	(SE)	M_B (SE)	X^2 (d.f.)
LSG	1975	1,667	0.028	0.090	0.31	(0.04)		
LSG	1976	2,028	0.043	0.090	0.48	(0.05)		
LSG	1978	278	0.025	0.090	0.28	(0.10)		
			Pooled LSG[c]				0.37 (0.03)	7.18 (2)**
HSG	1975	853	0.029	0.090	0.32	(0.06)		
HSG	1976	865	0.031	0.090	0.34	(0.07)		
HSG	1978	233	0.026	0.090	0.29	(0.11)		
			Pooled HSG[c]				0.33 (0.04)	0.21 (2)
			Pooled over orchards				0.36 (0.03)	0.83 (1)
Seed crop[d]	1975	486	0.041	0.090	0.456	(0.10)		
Seed crop[d]	1976	472	0.021	0.090	0.233	(0.07)		
							0.31 (0.06)	3.24 (1)
LSG (pollen)[e]	1978	278	0.025	0.042	0.50	(0.22)		
HSG (pollen)[e]	1978	233	0.026	0.042	0.61	(0.24)		

[a] Frequency of multilocus markers found in the orchard pollen pool
[b] Frequency of multilocus markers expected in background stands
[c] Weighted by variances
[d] Bulked LSG and HSG
[e] Based on pollen rather than genotype frequencies in the background stands

geneity test. It is larger, but not significantly so, than the HSG estimate for 1976 (0.34). A pooled estimate for the LSG is 0.37 over three years, and for the HSG, 0.33 over three years. These are not heterogeneous ($P > 0.05$), and an overall estimate of M_B is 0.36 ± 0.03. When the seed crop was analyzed, the estimate of gene flow was twice as great for 1975 as for 1976, although due to the large standard errors, these are not statistically significant differences. When an independence chi-square was used on the actual counts of contaminants, the seed crop sample in 1975 had significantly more contaminants than the 1975 ramet sample, and the seed crop in 1976 had significantly fewer contaminants than the 1976 ramet sample.

The simplest interpretation of these data is that different clones in the orchard produce differing amounts of seed, and that the clones also differ in their likelihood of being pollinated by trees outside the orchard. This would be most likely due to location in the orchard or phenology.

To investigate these possibilities, counts of contaminants were separated into clone and ramet within clone components using a partitioned chi-square. In the LSG, neither the clone nor the ramet within clone effect was statistically significant in 1975 or 1976. In the HSG orchard, the clone and ramet effects were confounded for each year because only one ramet was sampled of each clone. In both years, however, the ramets differed significantly ($P < 0.05$) in the number of contaminants they received. Clones or ramets can differ, therefore, in levels of contamination, and clones also differ in contributions to the seed crop (Friedman, in preparation). Nevertheless, when pooled over years, the bulk samples for two years (0.31) yield an estimate very close to the ramet samples for three years (0.36).

When the frequencies in the pollen pool of the background trees, rather than the gene frequencies of the trees themselves were used, the estimates of gene flow were approximately twice as large (0.60). This could only be done in 1978, because the trees were sampled and the gene frequencies in the male contribution to the offspring of the outside trees were obtained in that year. The large size of the estimates is probably because it was the relatively rare alleles that made up most of the multilocus combinations which did not occur in the orchards. These were generally present at lower frequencies in the pollen pool than they were in the outside trees.

Estimates of the proportion of seed from the LSG orchard fertilized by pollen from the HSG orchard based on the single-locus marker ranged from 0.07 to 0.16, with a pooled estimate for three years of 0.10 (Table 3). The estimates are in general agreement with those derived using the Smith and Adams (1983) methods which averaged 0.14. No variances were available for these estimates (Smith, personal communication). No single locus markers were available to detect gene flow from the LSG to the HSG, so the Smith and Adams (1983) method was the only possible estimation technique. These estimates averaged 0.09 for gene flow from the LSG orchard to the HSG.

Table 3. Estimated proportion (M_A) of progeny fertilized by pollen from the adjacent orchard based on two methods

Orchard	Year	Single locus marker M_A (s.e.)	Smith and Adams M_A
LSG	1975	0.13 (0.035)	0.16
	1976	0.07 (0.030)	0.05
	1978	0.16 (0.078)	0.21
Pooled over years		0.10[a] (0.022)	0.14
HSG	1975[b]		0.11
	1976		0.07
	1978		0.09
Pooled over years			0.09

[a] Weighted by variances
[b] No. single locus marker was available

Frequencies of the background stand multilocus markers observed in the pollen pool of each ramet were correlated with spatial and phenological variables, for 1975 and 1976. The only variable significantly related to proportion of contaminants received was the proportion of crown exposed to the outside, ($r = 0.55$, 20 d.f. $P < 0.01$) and only in 1976.

Discussion

Based on these estimates, gene flow from pollen of neighboring stands may be substantial in wind-pollinated forest trees. The fact that stands of loblolly pine extend for miles in every direction make it difficult or impossible to ascertain the source or sources of this gene flow. Nevertheless, the estimated 36% of pollen effective in fertilizing ovules which did not come from the sampled seed orchards did come from stands at least 122 m away.

The amount of gene flow measured here provides a possible mechanism for maintenance of genetic variability in sessile, long-lived organisms. The factors which influence gene flow are themselves subject to a great deal of variability. The relative amount of pollen production of different trees in different stands vary each year relative to each other. Local variation in climatic factors which affect pollen shedding and female receptivity can allow day-to-day and year-to-year differences in pollination patterns between stands. Phenology of female receptivity can vary in different

parts of the same tree, so that different pollen clouds can reach different parts of the same tree in one year. Wind direction can vary within one day and throughout pollination season, with varying amounts of turbulence. Therefore, it seems likely that both the amount and genetic composition of gene flow varies from year to year. In this study, the genotypes of outside trees were used as estimates of gene frequencies in the pollen involved in gene flow. Hence, any variation in genetic composition of the pollen cloud could not be detected or separated from variation in amount of gene flow.

The pollen pools of the trees in the background stands in the year in which they were sampled (1978) did not reflect the gene frequencies present in the sampled trees. The relatively rare alleles which were generally found in the multilocus combinations used to estimate gene flow were usually found at levels lower in the pollen pool than in the standing trees. This could be explained in two ways: 1) trees which contain these alleles were not effective in pollinating ovules in the proportion in which the trees occur, or 2) gene flow from unsampled stands with lower gene frequencies of these alleles was occurring into the sampled stands. If the first explanation is correct, the use of pollen pool frequencies in estimating gene flow is appropriate, because these estimates include the capability of different genotypes to pollinate ovules in a given year. If the second explanation is correct, then gene flow occurred into the sampled background stands from stands with even lower levels of these alleles than were found in the pollen pools. The source could be the seed orchards themselves, or a more general pollen cloud. Therefore, it appears that the estimate based on genotypes from the background stands (0.36) is more likely to be a low than a high estimate.

When the average orchard-to-orchard gene flow estimate (0.12) is added to that from background stands (0.36), it appears that at least 48% of seed in the orchards was fertilized by trees more than 100 m away.

The two methods used to calculate gene flow were in good agreement. Adding all the multilocus markers together leads to one overall contamination estimate, which is preferable to several group estimates. Dividing multilocus markers into groups was used to illustrate that the multilocus markers used generally produced similar contamination estimates. If each group of markers produced widely divergent estimates, the assumptions of the model would be called into question. A disadvantage of the Smith and Adams (1983) procedure was that sampling variances around the background stand gene frequencies were not used, so that the variance on M_B was underestimated, compared to the marker group procedure which used those variances. These variances could be added to the Smith and Adams approach. The orchard-to-orchard estimates provided by subtracting background from total contamination estimates were in good agreement with the single locus marker. As alleles which exist in only one orchard, and not in the background stands are very rare, this method provides the only opportunity to measure orchard-to-orchard or block-to-block contamination consistently. The Smith and Adams (1983) approach, then, is of great utility, but could be further improved by using the background stand gene frequency variances.

An unexpected finding was that the count of contaminants in the seed crop sample in 1975 was significantly greater in the ramet sample in 1975, and the count of contaminants in the seed crop sample in 1976 was significantly lower than the ramet sample in 1976. The clone with the highest proportion of contaminants in 1976 (0.08), was composed of two sampled ramets both of which were located on the edge of the LSG orchard. The next highest clone, however, with a frequency of 0.07, had two sampled ramets, neither of which was located near the edge of the orchard. The aforementioned frequencies 0.07 and 0.08 were actual proportions of the seed pollinated by trees with gene combinations not found in either orchard. No weighting by outside frequencies was done. If weighting were done similar to that in Table 2, 80 or 90% of the ovules from those clones would be estimated to have been pollinated by outside trees. Hence, it seems likely that both location and a phenology effect were involved in the differences among clones. The most likely reasons, then, for differences between the ramet samples and the seed crop samples were that certain ramets and clones were pollinated by more or fewer outside trees, and that these ramets and clones differed in seed production. The relative importance of phenology versus location could not be assessed due to inadequacies in the phenology data.

These results may be of value to seed orchard managers. Based on these findings it would appear that pollen contamination from background stands is likely to be a problem whenever a small orchard (4–6 ha) is surrounded by thousands of acres of forest stands of the same species. A meaningful reduction in contamination cannot be achieved by collecting seed from trees in the middle of orchards of this size, for phenology or other clonal characteristics are also involved in contmination. It may be possible to accomplish a reduction in contamination with a much larger dilution zone. There may be substantial differences in contamination between years in the same orchard (twofold, based on these data). Seed crops from different years could be assessed using these techniques, and those with the lowest contamination levels preferentially used. If it is desired to estimate pollen contamination in a given orchard, it would be important to sample the bulked seedlots themselves rather than a random sample of ramets due to possible differences in both seed production and pollen contamination by ramet and clone.

The difference between orchard-to-orchard gene flow estimates (0.12) and estimates from background stands (0.36) indicates that the amount of gene flow depends on the size, as well as the proximity, of the pollen source. The importance of contamination from both background stands and neighboring orchard blocks depends on at least two factors. First is the level of contamination. Second is the differential in growth

and economic traits between the two groups of trees involved, which increases substantially between untested and tested orchards, and first to second generation orchards. In the western U.S., different elevations are sometimes combined at one seed orchard site. The possibility then exists that surrounding stands and neighboring orchard blocks may not be adapted to the sites on which the seedlings from the seed orchard are to be planted. This could potentially lead to an actual decrease in growth and yield of seed orchard stock compared to reforestation stock from adapted unselected trees. Pollen contamination will also have an increasingly severe impact on advanced generation orchards (Sniezko 1981). Techniques to reduce pollen contamination, such as supplemental mass pollination, (Bridgewater and Trew 1982) may well be economically justified in such instances.

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