Proceedings from the Wood Compatibility Initiative Workshop, number 18.

COMPATIBILITY OF BREEDING FOR INCREASED WOOD PRODUCTION AND LONG-TERM SUSTAINABILITY: THE GENETIC VARIATION OF SEED ORCHARD SEED AND ASSOCIATED RISKS

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ABSTRACT

Because breeding imposes strong artificial selection for a narrow suite of economically important traits, genetic variation is reduced in seedlings derived from operational seed orchards. Both quantitative genetics theory and studies of allozyme variation show that seed orchards contain most of the genetic diversity found in natural populations, although low-frequency alleles are often absent from seed orchard populations. Because plantations established with seed orchard seed are frequently maintained for only a single generation, they do not need to preserve the low-frequency alleles that are maintained in the breeding and gene resource populations. Moreover, in the Pacific Northwest, low-frequency alleles are typically maintained in the breeding population and in *in situ* reserves that serve as gene resource populations. Our analysis of theoretical and empirical data indicates that seed orchards with 20 or more selections should provide the same level of risks as seed collected from the natural population.

KEY WORDS: Genetic diversity, tree breeding, seed orchards, risks.

INTRODUCTION

Genetic variation is essential for populations to be able to adapt to new stresses such as disease and climate change. The amount of genetic variation required for population viability is dependent on many factors, including the expected life of the population (e.g., rotation age), the number of future generations the population is expected to produce, the environmental variation (over time and space) to which the population must adapt, and the rate that mutation and migration adds genetic variation in the future. On one extreme are agronomic crops that are planted for a single generation lasting less than a year in a relatively uniform environment. On the other extreme are natural populations of forest species that are long-lived and are expected to adapt to changes in climate and environment for many centuries. Forest plantations tend to be somewhere in the middle of this continuum; they must survive for only a single generation, but generation intervals tend to be measured in decades, during which time they experience a variety of environments.

Many forest tree species have active, ongoing tree improvement programs. Breeding activities involve selection for heritable traits such as growth and yield, crown form, and wood quality. Tree improvement enables genetic gain but also may result in undesirable erosion of genetic variation that fosters adaptation and evolutionary success (Namkoong et al. 1988). Genetic variation may be lost at several steps in the tree improvement process including during the breeding cycle and establishment of production populations (i.e., seed orchards or clonal stool beds). Therefore, a question that continues to arise with all breeding programs is: Will the use of improved varieties result in a significant loss of natural genetic variation, hence reducing the long-term sustainability of species and ecosystems that depend on them? An additional question for forest trees concerns the viability of stands produced from seed orchards: Does the stand have sufficient genetic diversity to buffer short-term environmental pressures that it will encounter throughout a typical rotation cycle?

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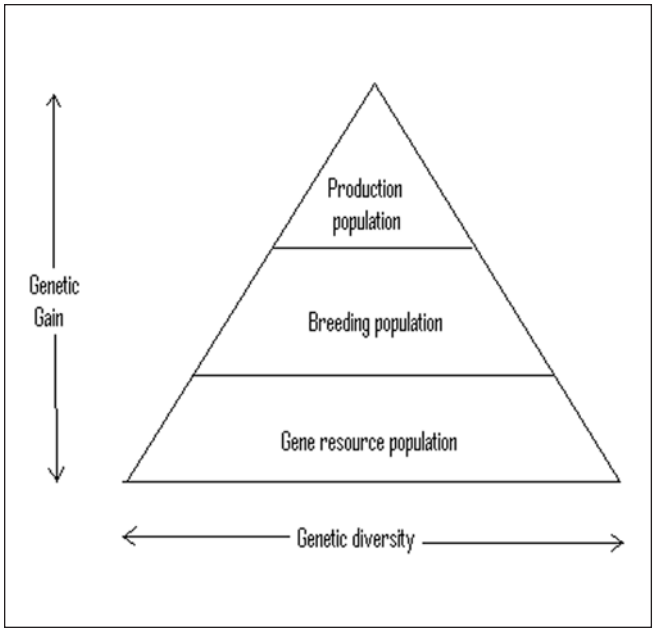

Figure 1—Conceptualization of gain versus genetic variation for a gene resource management program (from Burdon 1995[3])

The issues surrounding maintenance of genetic variation differ among the three populations that one must consider in a gene resource management strategy. Rowland Burdon (1995[3]) developed a pyramid that conceptualizes the role of various populations to consider in gene resource management (Figure 1). The horizontal axis represents genetic diversity and the vertical axis represents genetic gain, i.e. the level of genetic improvement. Each of these populations is associated with different scales of time and area.

The gene resource population represents all of the available genetic variation that could contribute to the species in future generations. This includes native stands, provenance trials, seed orchard parents, progeny in progeny tests, and operational plantations. Ideally, there will be sufficient genetic variation at this level to maintain the species indefinitely. Because the long-term (evolutionary scale) viability of the species (i.e., the gene resource population) is a major goal, thousands of parents are recommended to maintain the genetic variation at this level (Lande 1995, Lynch 1995, Millar and Libby 1991, Yanchuk 2001).

In the Pacific Northwest, the Pacific Northwest Forest Tree Gene Conservation Group has supported an effort to document the status of the gene resource and breeding populations of eight commercially important conifers (St. Clair and Lipow 2000). For all eight species (*Abies procera* Rehd., *Picea sitchensis* (Bong.) Carr., *Pinus ponderosa* Dougl. ex Laws., *Pinus lambertiana* Dougl., *Pinus monticola* Dougl. ex D. Don, *Pseudotsuga menziesii* (Mirb) Franco, *Thuja plicata* Donn ex D. Don, *Tsuga heterophylla* (Raf.) Sarg.), extensive and sufficient genetic resources are conserved *in situ* through much of the species regional range. However, for *Pinus monticola* and *Abies procera* there are a few local areas that may warrant additional gene conservation.

In the conceptualized gain:diversity pyramid (Figure 1), the next level is the breeding population; this group represents the individuals that will contribute their genes to the next generation in the breeding program. It tends to be more highly selected, and therefore potentially less variable, than the gene resource population. Breeding populations must have sufficient genetic variation to enable an attractive rate of progress in targeted traits for one or more generations. Suggested minimum population sizes range from tens to hundreds (see reviews by Johnson et al. 2001 and White 1992). For Douglas-fir, the predominant species being bred in the Pacific Northwest, advanced generation breeding programs are well within this range, with most breeding populations carrying over 200 individuals.

At the top of the gain:diversity pyramid is the production population, consisting of seed orchard parents or clones used for operational deployment. These genotypes are typically the best selections from the breeding population. This population must carry adequate genetic variation to sustain one rotation in an operational plantation and realize sufficient genetic gain to warrant the tree-breeding program. Because the primary purpose of the production population is to provide the best possible genetic material for operational planting, the number of selections must be limited, resulting in an unavoidable tradeoff between genetic variation and realized gain. Although maximizing genetic variation is a low priority in this population, substantial genetic variation is nearly always maintained in seed orchards, in the form of dozens or even hundreds of unrelated parent trees.

These three populations are not always different, and many species may not have active breeding and production populations. Or, for species that are in jeopardy of extinction (because of introduced pathogens or other causes), the production population may serve as the primary gene resource population.

[3] Presentation given at the Western Forest Genetics Association meeting, 28 August 1995, Victoria British Columbia.

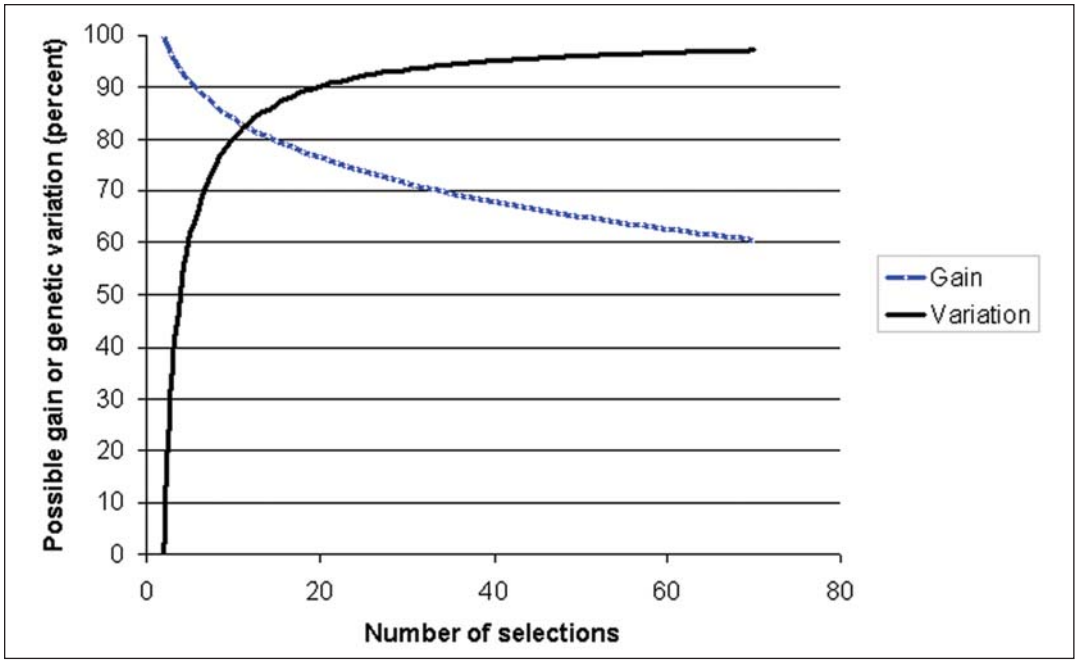

Figure 2—Example of genetic variation and genetic gain associated with seed orchards of differing numbers of clones. Genetic variation is modeled for Ne=N/2.

This paper reviews the literature that discusses the genetic variation found in seed orchards to help readers understand the risks–or lack thereof–associated with the use of genetically improved seed orchard seed.

THEORETICAL PREDICTIONS OF GENETIC VARIATION AND CAPTURE OF LOW-FREQUENCY ALLELES

Maintenance of genetic variation in any population, including the seed orchard, primarily involves the preservation of genetic diversity (i.e., heterozygosity) and, to a lesser extent, the maintenance of low-frequency alleles. Low-frequency alleles are valuable to breeding populations but have limited benefits to plantation forests. Low-frequency alleles are usually low frequency for a reason, resulting from deleterious mutations (as suggested in Adams et al. 1998, Schmidtling et al. 1999, Skrøppa 1996), or from past negative natural or artificial selection pressure, or from random drift (a function of sampling). For a trait that is controlled by many genes, each with a small effect, alleles at low frequency contribute little to overall genetic variation because they occur in a small percentage of individuals. Even low frequency alleles with potentially major beneficial effects (e.g., those that confer disease resistance) are of little value in a seed orchard because they could only benefit a small percentage of the trees in resulting plantations. Such alleles are of much more value in the breeding population where their frequency can be increased to the benefit of future production populations.

Theoretical Calculation of Genetic Variation

Population genetics theory makes it possible to predict how well a given number of selections capture the genetic variation of a given population. Additive genetic variation is that portion of the total genetic variation that responds to natural and artificial selection. In forest trees, additive genetic variation makes up the largest component of the total genetic variation and is more affected by population size than by nonadditive genetic variation; therefore, a reduction in additive genetic variation provides a conservative estimate of the reduction in the total genetic variation. The formula to calculate the percentage of total additive genetic variation ($\%V_A$) in a seed orchard relative to the natural population from which it is derived is:

$$\%V_A = 100 \times [1- 1/(2N)],$$

where N is the number of selections. For obligate outcrossing and monoecious species the equation is modified to:

$$\%V_A = 100 \times [1- 1/(N)]$$

(Johnson 1998).

Studies indicate that truly random mating (panmixis) does not happen in the seed orchard, because 80 percent of the seed is typically produced by 20% of the clones (Anon. 1976). Although various factors contribute to the unequal reproductive contribution of parents (reviewed by El-Kassaby 2000), they all reduce overall genetic variation in the resulting seed orchard seed. This reduction can be evaluated by examining the effective population size in the seed orchard. Effective population size (Ne) is defined as the number of individuals that would give rise to the genetic variance or inbreeding coefficient if mating were actually panmictic (Falconer and Mackay 1996). Estimates of the effective population sizes of seed orchards relative to the census number of clones (N) or families vary widely, making it difficult to generalize as to the extent to which genetic variation in the seed orchard crop is reduced relative to the orchard selections. Most of estimates do suggest that the ratio of Ne/N is greater than 0.5, although within an orchard, there appears to be considerable variation among years (El-Kassaby and Askew 1991, Kjaer 1996). By assuming Ne = N/2, and substituting Ne in the above equation, we can obtain a conservative estimate of the additive genetic variation coming from a seed orchard (Figure 2). A seed orchard with 25 unrelated selections contains about 92 percent of the genetic variation of the natural population. In short, theory suggests that seed derived from an orchard of reasonable size (20 or more clones) will harbor most of the genetic variation found in the native population.

Figure 2 demonstrates the relation between genetic gain and genetic diversity for an obligate outcrossing species after substituting N with an estimate of Ne (where Ne=N/2). Gain increases sharply as selection numbers fall below 30 and genetic diversity increases sharply up to about 20 selections. This tradeoff must be considered when planning a seed orchard.

Probability of capturing rare alleles—The probability rare alleles will be lost in a seed orchard with a given number of selections can be calculated. For an outcrossing species, the probability that an allele of frequency p found in the natural population does not occur in the orchard is $(1-p)^{2N}$, where N equals the number of clones in the orchard. This equation suggests that, in general, a seed orchard with 25 clones will include all alleles with frequency > 0.05 with a 92 percent probablity. Again, theory predicts that most alleles will be included in a seed orchard of moderate size.

Direct Measures of Genetic Variation Coming from Seed Orchards

Despite the theoretical predictions that a seed orchard contains a representative portion of the genetic variation found in natural populations, the concern that diversity in seed orchards may be compromised under intense breeding and selection schemes has impelled several empirical studies (Table 1). These studies compare genetic variation within seed orchards with genetic variation found in natural populations from the same or a larger source region. They evaluate presumably neutral allozyme loci and then estimate several population genetic statistics that allow for comparison (Berg and Hamrick 1997). These include:

- Percentage of polymorphic loci (%P) –the percentage of loci that have more than one detected allele. The %P is partly a function of the population sample size, because low-frequency alleles are more likely to be observed in larger samples.

- Allelic diversity–the mean number of alleles (A) and the mean number of alleles per polymorphic locus (A_p).

- Expected proportion of heterozygous loci per individual (H_e)–reflects the heterozygosity that would be expected in a population experiencing Hardy-Weinberg equilibrium. It is a function of the proportion of polymorphic loci, the number of alleles per polymorphic locus, and the evenness of allele frequencies within population. It is computed as

$$H_e = 1 - \sum p_i^2,$$

where p_i is the mean frequency of the ith allele and is averaged over all loci.

- Observed heterozygosity (H_o) is a related statistic. It is figured directly from observed genotype frequencies and thus is affected by inbreeding and other evolutionary processes.

Studies Confirm Similar Genetic Variation in Seed Orchards and Natural Populations

Table 1 summarizes values of %P, A_p, A, H_e, and H_o from 14 allozyme studies comparing genetic variation between first-generation seed orchards and natural populations. The general conclusion from these studies is that seed orchards retain most of the genetic variation present in the natural populations from which they were derived, although, as predicted, some low-frequency alleles or alleles that were restricted to only one or a few populations are not included.

In most studies, the values of %P and allelic diversity (A and A_p) did not differ substantially between the means of the natural populations and the seed orchards. This is exactly as theory predicts. Moreover, the capture of genetic variation in the seed orchards was likely enhanced by

differences in sampling methods between the natural populations and the seed orchards. Sampling of natural populations was typically restricted to a single stand, whereas the selections in seed orchards were usually chosen from numerous stands, adding additional opportunities for picking up alleles. Indeed, when selections in a Sitka spruce orchard were sampled across an atypically large area, the %P and A were actually higher in the orchard than in any one sampled natural population (Chaisurisri and El-Kassaby 1994). Additionally, contamination by alien pollen led to higher allelic diversity in the seed produced by an interior spruce orchard relative to the mean of the natural populations (Stoehr and El-Kassaby 1997). Pollen contamination also may have been responsible for the novel alleles detected in the seed orchards of the other species (El-Kassaby and Ritland 1996b, Godt et al. 2001).

When pooled results from natural populations were examined, as opposed to mean values, overall reduced allelic diversity (%P, A, and A_p) in the seed orchard selections relative to the source region was revealed; however, measures of heterozygosity (H_e and H_o) were still similar. The reduction in allelic diversity is not surprising as the number of samples from the pooled natural populations were, on average, twice the number of selections from the orchards. The few cases of lower H_e in the orchard were ascribed to missing low-frequency alleles (Knowles 1985, Moran and Bell 1987, Stoehr and El-Kassaby 1997). Despite some slight variations in patterns of heterozygosity, these combined results again confirm that only a minor reduction of genetic diversity results when selections are chosen for a seed orchard.

Genetic Diversity in Rogued First-Generation and Advanced-Generation Seed Orchards

Genetic testing in tree improvement enables the breeding values of selections in a breeding population to be determined. Once breeding values have been calculated, first-generation seed orchards can be rogued to leave only the best selections, or second-generation seed orchards can be developed. This process leads to a reduction in the number of parent trees within the seed orchard and, unavoidably, the loss of some genetic variation.

Only one study has directly compared genetic variation in seed orchards at various stages of improvement. El-Kassaby and Ritland (1996b) examined allozyme diversity in 12 first-generation Douglas-fir seed orchards before and after roguing, as well as in four second-generation seed orchards. They report that H_e, %P, and A did not differ significantly among the various orchard populations. Nevertheless, at each stage in the domestication process, some rare and local alleles were lost. These results are backed up by two studies of simulated roguing of first-generation seed orchards (Godt et al. 2001, Stoehr and El-Kassaby 1997), both of which show that expected heterozygosity remains high even if up to 50 percent of the orchard parents are removed. The number of alleles per locus drops more rapidly with roguing than does expected heterozygosity, as would be expected with any genetic bottleneck.

RISKS

A reduced range of genetic variation could potentially result in incremental damage and mortality in plantations if the genes "lost" or under-represented are related to traits that turn out to be important in the environments that the plantation has to face over its lifetime–although the "low-frequency" alleles typically lost would be unlikely to have this kind of substantive effect. Still, with the advent of clonal forestry operations, analyses have been made to estimate the risk potential caused by decreased genetic diversity in clonally propagated plantations. Several assumptions are involved in these estimates, but in general, the number of clones needed to give levels of risk similar to natural populations ranged from 17 to 40, depending on the analysis (Bishir and Roberds 1995, Hühn 1986, Libby 1982, Roberds and Bishir 1997, Roberds et al. 1990). These analyses indicate there should be little increase in risk associated with the level of genetic variation in seed lots from most orchards.

An argument is also sometimes made that the faster growing families typically represented in a seed orchard, although adapted to the climate of the planting zone, may be less resistant to diseases and pests. This argument is based on the premise that there is a physiological tradeoff between growth and defense (Herms and Mattson 1992), and has been demonstrated in comparisons among some species. However, this trend has not been established when one looks at families within a breeding zone. Typically, the opposite is true: empirical studies frequently show that the faster growing families tend to be the healthier families. In Douglas-fir, favorable genetic associations have been shown for growth and resistance to Swiss needle cast (Johnson and Temel 1998), and for growth and terpine content (Kimball et al. 1999), a deterrent to bear damage. Other resistant traits having favorable genetic (or family mean) correlations with growth include: foliage disease (*Cyclaneusma minus* (Butin) Di Cosmo, Peredo and Minter) in *Pinus radiata* D. Don (King et al. 1998), fusiform rust (*Cronartium quercuum* (Berk) Miyabe ex Shirai f. sp. *fusiforme*) (McKeand and Bridgwater 1995) and tip moth

Table 1—Means and population ranges (in parenthesis) of percentage of polymorphic loci (%P), mean number of alleles (A), mean number of alleles per polymorphic locus (A_p), expected proportion of heterozygosity (H_e), and observed heterozygosity (H_o) from seed orchards (SO) and the natural populations (NP). (continued)

Species	Population	Number of sample trees (N)	%P	A	A_p	H_e	H_o	Citation
Picea abies (L.) Karst	1 SO	45	88	2.37			0.23	Bergmann and Ruetz 1991
	3 NP pooled	60	100	2.5			0.32	
	3 NP mean	60	83.7 (75-88)	2.21 (2.0-2.37)			0.33 (0.22-0.24)	
Picea glauca (Moench) Voss	1 SO	40	50	1.72	2.44	0.157		Godt et al. 2001
	7 NP pooled	~336	55.6	2.17	3.1	0.164		
	7 NP mean	~336	46.8 (44-56)	1.72 (1.61-1.83)	2.55 (2.38-2.88)	0.161 (0.157-0.166)	0.163 (0.153-0.181)	
Picea glauca x engelmanni	1 SO	100	64.7	2.4		0.207	0.194	Stoehr and El-Kassaby 1997
	9 NP pooled	360	64.7	2.7		0.21	0.189	
	9 NP mean	360	62.73 (53-71)	2.16 (1.9-2.1)		0.203 (0.154-0.223)	0.189 (0.150-0.226)	
Picea mariana (Mill) Britton et al.	1 SO	58	100	2.8		0.327	0.272	Knowles 1985
	2 NP mean	201 (100-101	100	2.9 (2.8-3.0) (2.8-3.0)		0.329 (0.321-0.336)	0.32 (0.296-0.345)	
Picea sitchensis (Bong) *Carriere*	SO	134	100	2.77		0.229		Chaisurisri and El-Kassaby 1994; Yeh and El-Kassaby 1980
	10 NP mean	782	66.92 (54-85)	1.82 (1.62-2.00)		0.183 (0.159-0.207)		

Table 1—Means and population ranges (in parenthesis) of percentage of polymorphic loci (%P), mean number of alleles (A), mean number of alleles per polymorphic locus (A_p), expected proportion of heterozygosity (H_e), and observed heterozygosity (H_o) from seed orchards (SO) and the natural populations (NP). (continued)

Species	Population	Number of sample trees (N)	%P	A	A_p	H_e	H_o	Citation
Pinus banksiana Lamb.	SO	31	44	1.67	2.5	0.114		Godt et al. 2001
	5 NP pooled	~240	59.3	2.04	2.75	0.114		
	5 NP mean	~240	46.7 (41-52)	1.66 (1.59-1.74)	2.41 (2.33-2.50)	0.112 (0.097-0.127)		
Pinus caribaea Morelet var *caribaea*	1 Seed Orchard	109	62.5	2.3		0.274		Zheng and Ennos 1999
	5 NP mean	80	60.0 (50-75)	2.26 (2.1-2.3)		0.272 (0.250-0.297)		
Pinus palustris Mill	3 Seed Orchards - mean	30	57.6 (45-68)	1.88 (1.59-2.05)		0.112 (0.101-0.129)	0.111 (0.096-0.122)	Schmidtling and Hipkins 1998
	20 NP plus the 3 SO mean	618	62.1 (50-86)	1.92 (1.50-2.27)		0.105 (0.075-0.147)	0.103 (0.072-0.144)	
Pinus radiata D. Don	20 Seed Orchard pooled	162	77.4	2.16		0.092		Moran and Bell 1987
	20 Seed Orchard mean	162	48.4 (39-55)	1.58 (1.45-1.65)	0.091 (0.083-0.097)	0.089 (0.079-0.097)		
	5 NP pooled	a	87.1	2.39		0.117		
	5 NP mean	a	56.8	1.79		0.098		
Pinus sylvestris L.	2 Seed Orchards - pooled			3.7		0.37		Rudin et al.1974 and Rudin and Lindgren 1977

Table 1—Means and population ranges (in parenthesis) of percentage of polymorphic loci (%P), mean number of alleles (A), mean number of alleles per polymorphic locus (A_p), expected proportion of heterozygosity (H_e), and observed heterozygosity (H_o) from seed orchards (SO) and the natural populations (NP). (continued)

Species	Population	Number of sample trees (N)	%P	A	A_p	H_e	H_o	Citation
	2 Seed Orchards - mean			3.5		0.36		compared in Adams 1981
	3 NP pooled			5.3		0.38		
	3 NP mean			4.3		0.37		
Pinus sylvestris L.	3 SO mean	1227[b]			2.66 (2.38-2.92)	0.276 (0.258-0.305)	0.229 (0.214-0.245)	Szmidt and Muona 1985
	3 NP mean	401[b]			2.84 (2.69-2.92)	0.294 (0.257-0.316)	0.249 (0.237-0.262)	
Pseudotsuga menziesii (Mirb.) Franco Oregon	1 SO	77		3	0.247			Adams 1981
	4 NP pooled	97		3.1		0.233		
Pseudotsuga menziesii (Mirb.) Franco Washington	1 SO	79		2.8		0.239		Adams 1981
	4 NP pooled	88		3	0.249			
Pseudotsuga menziesii (Mirb.) Franco British Columbia	12 Seed Orchards pooled	936	62.5[c]	2.28		0.172		El-Kassaby and Ritland 1996a, 1996b
	(1st generation, unrogued)	(54-114)						
	12 SO pooled	731	60.4	2.24	0.173			
	(1st generation rogued)	(37-94)						
	4 SO pooled	223	56.3	2.25		0.163		

Table 1—Means and population ranges (in parenthesis) of percentage of polymorphic loci (%P), mean number of alleles (A), mean number of alleles per polymorphic locus (A_p), expected proportion of heterozygosity (H_e), and observed heterozygosity (H_o) from seed orchards (SO) and the natural populations (NP).

Species	Population	Number of sample trees (N)	%P	A	A_p	H_e	H_o	Citation
	(2^{nd} generation)	(40-85)						
	49 NP pooled	2497	85	3.05		0.163		
	49 NP mean	2497	52.6 (40-70)	2.14 (1.75-2.35)		0.171 (0.122-0.198)		
		(43-61 per population)						

[a] 300 progeny per population with 50 families X 6 progeny.
[b] In Szmidt and Muona 1985 embryos and not adult tissue were examined in both the SO and NP. Also, 3 seed crops from 3 different years were studied per SO.
[c] El-Kassaby and Ritland (1996a, 1996b) considered a locus to be polymorphic only if the frequency of the most common allele was less than 0.95.

(*Rhyacionia frustrana* Zimm.) tolerance (Hedden et al. 2001) in *Pinus taeda* L. and weevil (*Pissodes strobi* (Peck)) resistance in a British Columbia population of interior spruce (*Picea sp.*) Carr (King et al. 1997). (Care must be taken, however, to consider adaptive traits [such as cold and drought hardiness] when selecting from outside of established seed or breeding zones.) Again, the potential impact of genetic variation lost through selection does not appear to increase risk in use of improved orchard progeny.

CONCLUSION

The theoretical and measured amounts of genetic diversity coming from seed orchards are in agreement. Although seed orchards do not capture all the low-frequency alleles in a population, they do have most of the genetic variation. Both for loss of random genetic diversity (reduction in numbers) and for diversity lost through directional selection, a seed orchard population size of 20 or greater will have over 90 percent of the genetic variation found in the entire population. When used in their native breeding zone (or even when used after testing outside their breeding zone), progeny from seed orchards should have risk properties comparable to natural populations.

ACKNOWLEDGMENTS

We thank Rich Cronn, Nancy Mandel, Frank Sorensen, Cheryl Talbert, and Alvin Yanchuk for their helpful comments.

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