REGENERATION METHODS AFFECT GENETIC VARIATION AND STRUCTURE IN SHORTLEAF PINE (*PINUS ECHINATA* MILL.)[1]

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ABSTRACT

The effects of regeneration methods on genetic diversity and structure in shortleaf pine (*Pinus echinata* Mill.) were examined by quantifying the changes in genetic composition of shortleaf pine stands following harvest by monitoring changes in allele number and frequency at heterozygous loci over time. The results were also compared to the genetic composition of seed used for artificial regeneration following clear-cutting. Both natural regeneration treatments resulted in higher genetic variation in post-treatment seed, indicating a richer pollen cloud after management. Artificial regeneration showed fewer alleles per locus and fewer polymorphic loci compared to both natural regeneration treatments. Frequency of alternate alleles increased at 13 loci in the seed-tree stand after treatment, which is an indication of less inbreeding or consanguineous mating. Single tree selection resulted in an increase in alternate allele frequencies at 9 loci and at 4 loci alternate allele frequencies decreased, indicating that the treatment may result in more inbreeding than seed tree. Artificial regeneration showed a considerable increase in alternate allele frequencies at 16 loci and hence can be considered outbred. The above mentioned observations were confirmed by comparing H_o, H_e and F values for the two stands before and after treatment. The seed tree method resulted in a decrease in inbreeding, whereas the first selection cut for single tree selection did not alter it. Artificial regeneration showed a negative F value indicative of high levels of heterozygosity and outbreeding. The natural regeneration treatments did not result in genetic drift whereas the artificial regeneration showed considerable change in the genetic composition of the potential regeneration.

Key words: isoenzyme, genetic variation and structure, seed-tree, single tree selection, natural and artificial regeneration, shortleaf pine, *Pinus echinata*

INTRODUCTION

Increased public desire to maintain genetic diversity in forests has resulted in a growing concern over the influence of forest management practices on genetic variation in forests. Continuing demand for forest products, the increasing demand for use of forested areas for non-traditional purposes, and the general public desire to maintain landscape diversity, biodiversity, conserve wildlife, protect old growth forests, control ecodegradation and global climate change has put a complex array of often conflicting demands, priorities and conditions on forest managers. The choice of suitable management strategies applicable to the climatic, political and public situation of the forests under their care has become exceedingly difficult. Therefore, an evaluation of within-species genetic diversity of existing stands compared to that of stands regenerated by various management schemes would help in understanding man's effect on these stands, and may suggest suitable management strategies.

The trend on federal lands has been to move from artificial regeneration methods like clear-cutting to natural regeneration systems such as seed-tree and single tree selection methods. The genetic consequences of various natural and artificial regeneration methods have been hypothesized (DANIEL *et al.* 1979) but there have been very few efforts to quantify changes in genetic variation at a molecular level.

NEALE and ADAMS (1985) studied the mating system of an uncut and a shelterwood stand of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in Oregon. Their results suggested related matings other than selfs probably are occurring in uncut stands but not in shelterwoods. However, since they were not able to detect significant differences, they proposed an expanded study of this nature with lower residual stand densities. Two studies in Europe evaluating genetic changes during different life stages in Scots pine (*Pinus sylvestris* L.) found that inbreeding was reduced from

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12% in embryos to less than 1% in 3-year old natural regeneration established from those seeds (MUONA *et al.* 1987) and that excess homozygosity found in embryos disappeared from the surviving regeneration by age 10–20 (YAZDANI *et al.* 1985), both indicating that elimination of inbred individuals occurs during stand establishment and early competition.

A study of change in population structure in a parent and adjacent progeny stand of loblolly pine (*Pinus taeda* L.) (ROBERDS & CONKLE 1984) found that although allele frequencies did not differ between the parent and progeny stands, genetic population structure was not the same, demonstrating that local genetic structure can differ between successive generations in a stand. Such a change may occur due to various regeneration methods and is the kind of knowledge required to address the question of management effects on genetic diversity. Studies comparing genetic variation and heterozygosity in seed orchards and natural stands show contradicting results. BERGMANN and RUETZ (1991) reported no change in gene frequency distributions (percentage of polymorphic loci p, or mean number of alleles per locus A), but found higher levels of heterozygosity in seed orchard trees compared to natural stands, whereas CHAISURISRI and EL-KASSABY (1994) reported significantly higher values for p and A for seeds from seed orchards, but the mean heterozygosities were not significantly different.

These studies suggest certain trends regarding the genetic consequences of management but do not give a clear picture since methods and results were variable. In this study, we report the changes in genetic variation and structure at 31 isoenzyme loci in two shortleaf pine stands managed by the seed-tree and single tree selection systems. The results are also compared to the potential genetic variation that would be found in these stands had they been artificially regenerated using bulked seed from two different seed orchards. Changes in the genetic composition of regeneration following management is expected because the stand density and thereby the genetic pool is altered due to management.

We chose the seed-tree and single tree selection systems for our study since they are commonly in use and represent the two extremes of selection pressure, seed-tree being most intense with residual pine basal area of 3.7 $m^2 \cdot ha^{-1}$, and single tree selection being the least with residual pine basal area of 14.2 $m^2 \cdot ha^{-1}$ (WITTWER *et al.* 1997).

MATERIALS AND METHODS

Plant material, electrophoresis and enzyme detection procedures

Seeds from 48 trees in each of two 15-hectare shortleaf pine stands in the Ouachita Mountains of Montgomery County, Arkansas were collected in the fall of 1993. Approximately 70% of the basal area in these stands was shortleaf pine and 30% deciduous species, predominantly *Quercus* and *Carya* species (WITTWER *et al.* 1997). The average age of dominant shortleaf pine trees in both stands was 64 years. Each stand was subdivided into quarters of approximately equal area arranged perpendicular to the elevation gradient and each quarter further subdivided into thirds along the elevation gradient, as part of a large ecosystem management research study on the Ouachita and Ozark National Forests in west-central Arkansas and eastern Oklahoma (GULDIN *et al.* 1993). A plot center was marked in each of the 12 subdivisions and 4 healthy trees of at least 20 cm diameter at breast height with abundant cones were selected in each subdivision for this study. Seed-tree and single tree selection harvest / regeneration systems were applied to the two stands, respectively, about three months before the first seed collection. Two years later the seed crop was collected representing the genetic variation after management was imposed.

The seed samples were assayed to detect changes in genetic variation due to management. Twenty-five seeds from each of the 48 trees from each stand for both pre- and post-treatment were assayed for 34 isoenzyme loci that were found polymorphic through an earlier study (RAJA *et al.* 1997). Fifty seeds each from the bulked seeds of the Ouachita and Ozark seed orchards were also analyzed to represent artificial regeneration for these stands, had they been clear-cut and planted with seedlings from those seed orchard seeds. A sample of fifty seeds from each seed orchard is adequate to enable meaningful comparisons with the data obtained from the seed-tree and single tree selection systems since these seeds originate from a limited number of clones in a seed orchard. Seed extraction and storage procedures, sample preparation, starch gel electrophoresis, enzyme staining and isoenzyme detection procedures followed protocols described by RAJA *et al.* (1997). Thirty-four loci belonging to 20 enzyme systems were assayed, from which 31 loci were resolved and consistently scorable in this study. The enzyme systems were aconitase (ACO, EC 4.2.1.3, 1 locus), acid phosphatase (ACP, EC 3.1.3.2, 2 loci), adenylate kinase (ADK, EC 2.7.4.3, 2 loci), alcohol dehydrogenase (ADH, EC 1.1.1.1, 1 locus), aldolase (ALD, EC 4.1.2.13, 2 loci), diaphorase (DIA, EC 1.6.4.3, 1 locus), glutamic dehydrogenase (GDH, EC 1.4.1.3, 1 locus), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1, 2 loci), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49, 2 loci), glycerate-2-dehydrogenase (G2D, EC 1.1.1.29, 1 locus), isocitric dehydrogenase (IDH, EC 1.1.1.42, 1 locus), malic

dehydrogenase (MDH, EC 1.1.1.37, 4 loci), malic enzyme (ME, EC 1.1.1.40, 1 locus), menadione reductase (MNR, EC 1.6.99.2, 2 loci), phosphoglucose isomerase (PGI, EC 5.3.1.9, 1 locus), phosphoglucomutase (PGM, EC 2.7.5.1, 1 locus), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44, 2 loci), sorbitol dehydrogenase (SDH, EC 1.1.1.14, 1 locus), shikimate dehydrogenase (SKDH, EC 1.1.1.25, 2 loci) and uridine diphosphoglucose pyrophosphorylase (UGPP, EC 2.7.7.9, 1 locus).

DATA ANALYSIS

Megagametophytes and embryos from each seed were scored for each locus. Identification of pollen genotype was accomplished by comparing megagametophyte and embryo data. Haploid pollen allele frequencies and diploid embryo genotypic frequencies were then calculated. Pollen allele frequencies for pre-treatment and post-treatment, and pre-treatment and artificial regeneration were compared with χ^2 tests (SNEDECOR & COCHRAN 1967, p. 250). When expected values were too small for χ^2 tests, Fisher's exact test was used (SOKAL & ROHLF 1981, p. 740). Genetic diversity was estimated by percent polymorphic loci 'p', mean number of alleles per locus 'A' and mean number of alleles per polymorphic locus 'A_p'. Diploid embryo data from each stand were pooled for pre-treatment, post-treatment, and artificial regeneration to calculate the observed (H_o) and expected (H_e) heterozygosities, and the fixation index using the formula :

$$F = 1 - (H_o/H_e) \qquad [1]$$

Levels of genetic differentiation between stands were estimated using Wright's F statistics, F_{ST} (WRIGHT 1965; 1969; 1978; NEI 1977). Haploid pollen allele frequencies, diploid embryo genotypic frequencies, p, A and A_p were calculated using the FREQ procedure of SAS computer program, H_o and H_e using BIOSYS-1 computer program (SWOFFORD & SELANDER 1981), and F_{ST} using GDA computer program (WEIR 1996).

RESULTS AND DISCUSSION

Genetic Diversity

The 20 enzyme systems assayed identified 75 electrophoretic variants at 31 loci. Twenty nine of the 31 loci assayed exhibited polymorphism in at least one stand. Seven of the 75 electrophoretic variants found were seen only in one stand, 4 in the seed-tree stand (at loci *Adh*, *Adk–2*, *Ald–2* and *G6pd–1*) and 3 in the single tree selection stand (at loci *Acp–1*, *Mdh–4* and *Sdh*).

Table 1 presents the haploid pollen allele frequencies by locus with c^2 and probability values for testing allele frequency differences between pre- and post-treatment for both the seed-tree and single tree selection methods, as well as for the two pre-treatment stands with artificial regeneration. The seed-tree method resulted in a gain of 8 alleles at 7 loci (*Adh*, *Adk–2*, *G6pd–1*, *G6pd–2*, *Got–1*, *6Pgd–2* and *Pgi*) and a loss at one (*Mdh–4*) following treatment. The single-tree selection method resulted in a gain of 9 alleles at 7 loci (*Acp–1*, *Adh*, *Got–1*, *Got–2*, *Mdh–1*, *Mdh–4* and *Me–1*) and a loss at one (*Sdh*). Artificial regeneration resulted in a gain of 6 alleles at 6 loci (*Acp–1*, *Adh*, *Got–1*, *Got–2*, *Mdh–1* and *Me–1*) and a loss of 13 alleles at 11 loci (*Acp–2*, *Ald–2*, *G6pd–1*, *G6pd–2*, *Mdh–1*, *Mdh–3*, *Mdh–4*, *Me–1*, *6Pgd–2*, *Pgi* and *Sdh*). It is important to note that all results reported here are based on the seed available for regeneration of these stands, and may not necessarily represent the genetics of advanced regeneration.

The seed-tree and the single tree selection system resulted a similar increase in P (about 8%), A (about 12%) and A_p (about 8%) following treatment (Table 2). However, the absolute values of P and A were consistently lower for the single-tree selection system compared with the seed-tree system. It is to be noted that the lowest values for P, A and A_p were observed for the seeds representing artificial regeneration (Table 2).

By comparing the change in number of alleles, and the genetic diversity estimates P, A and A_p, it can be inferred that the seed-tree and single tree selection systems resulted in a richer pollen cloud after treatment whereas seed orchard for artificial regeneration had a less diverse pollen cloud. The data also confirms that meaningful comparisons between the two natural regeneration systems and the artificial regeneration is possible from this study since comparable levels of gain in alleles were detected in these systems in spite of the sample size differences.

Genetic Structure

In addition to the loci that gained alleles in the various treatments as mentioned above, a significant increase in the frequency of alternate alleles (less frequent allele/s at a locus) following treatment was observed at 13 loci in the seed-tree stand, at 9 loci in the single tree selection stand, and at 16 loci in the seed representing artificial regeneration (Table 1). It is interesting to note that while the frequency of alternate alleles was not reduced at any loci in the seed-tree stand and the artificial regeneration, a significant reduction was observed at 4 loci in the single tree selection stand. The significance of an increase in the frequency of al-

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Table 1. Pollen allele frequencies (F) by locus for seed-tree, single tree selection, and artificial regeneration. χ^2 values and significance levels (*P*) for testing pre- (PR), post-treatment (PS) and artificial regeneration allele frequency differences are also presented.

		Treatment												
Locus	Allele	Seed-tree				Single tree selection				Artificial regeneration				
		F(PR)	F(PS)	χ^2	*P*	F(PR)	F(PS)	χ^2	*P*	F	χ^2	P(1)[a]	χ^2	P(2)[b]
Aco	A	0.073	0.110	8.29	<0.01*	0.099	0.093	0.21	0.65	0.130	4.15	0.04*	0.92	0.34
	B	0.927	0.890			0.901	0.907			0.870				
Acp–1	A	0.005	0.016	6.59	<0.01*	–	0.006	16.08	<0.01**	0.060	28.9	<0.01*	50	<0.01**
	B	0.995	0.984			1.000	0.980			0.940				
	C	–	–			–	0.014			–				
Acp–2	A	0.003	0.013	21.27	<0.01*	0.024	0.048	16.07	<0.01*	–	159.9	<0.01*	56.12	<0.01*
	B	0.994	0.964			0.948	0.944			0.810				
	C	0.003	0.023			0.028	0.008			0.190				
Adh	A	–	0.012	13.92	<0.01**	–	0.001	0.98	0.32**	0.010	10.36	<0.01**	8.62	<0.01**
	B	1.000	0.987			1.000	0.999			0.990				
	C	–	0.001			–	–			–				
Adk–1	B	1.000	1.000	–	–	1.000	1.000	–	–	1.000	–	–	–	–
Adk–2	A	–	0.003	2.19	0.14**	–	–	–	–	–	–	–	–	–
	B	1.000	0.997			1.000	1.000			1.000				
Ald–1	B	1.000	1.000	–	–	1.000	1.000	–	–	1.000	–	–	–	–
Ald–2	A	0.001	0.002	0.33	0.57	–	–	–	–	–	0.1	0.76	–	–
	B	0.999	0.998			1.000	1.000			1.000				
Dia	A	0.044	0.138	50.3	<0.01*	0.021	0.107	49.43	<0.01*	0.200	39.97	<0.01*	72.37	<0.01*
	B	0.956	0.862			0.979	0.893			0.800				
G2d	A	0.489	0.488	0.002	0.96	0.440	0.440	0	1	0.510	0.16	0.69	1.77	0.18
	B	0.511	0.512			0.560	0.560			0.490				
G6pd–1	A	–	0.007	7.01	0.03**	0.004	0.015	5.95	0.02*	–	0.7	0.4	0.36	0.54
	B	0.993	0.986			0.996	0.985			1.000				
	C	0.007	0.007			–	–			–				
G6pd–2	A	–	0.006	6.15	0.01**	0.006	0.002	1.41	0.24	–	–	–	0.61	0.44
	B	1.000	0.994			0.994	0.998			1.000				
Gdh	A	0.072	0.071	0.02	0.9	0.086	0.082	0.11	0.74	0.080	0.09	0.77	0.04	0.84
	B	0.928	0.929			0.914	0.918			0.920				
Got–1	A	–	0.019	133.1	<0.01**	–	0.008	51.71	<0.01**	0.060	143.8	<0.01**	82.62	<0.01**
	B	0.998	0.871			0.986	0.910			0.840				
	C	0.002	0.110			0.014	0.082			0.100				
Got–2	A	0.002	0.043	62.35	<0.01*	–	0.042	60.76	<0.01**	0.220	196.3	<0.01*	177.8	<0.01**
	B	0.794	0.665			0.775	0.627			0.540				
	C	0.204	0.292			0.225	0.331			0.240				
Idh	A	0.056	0.058	0.064	0.8	0.041	0.058	2.92	0.09	0.040	0.44	0.51	0.001	0.98
	B	0.944	0.942			0.959	0.942			0.960				

Table 1. (continued)

Locus	Allele	Treatment												
		Seed-tree				Single tree selection				Artificial regeneration				
		F(PR)	F(PS)	χ^2	*P*	F(PR)	F(PS)	χ^2	*P*	F	χ^2	P(1)[a]	χ^2	P(2)[b]
Mdh–1	A	0.994	0.995	2.28	0.32	1.000	0.997	2.85	0.24**	0.960	32.92	<0.01*	34.58	<0.01**
	B	0.001	0.003			–	0.002			0.040				
	C	0.005	0.002			–	0.001			–				
Mdh–2	A	0.009	0.027	16.32	<0.01*	0.022	0.012	7.67	0.02*	0.010	9.22	<0.01*	10	<0.01*
	B	0.987	0.956			0.974	0.974			0.960				
	C	0.004	0.017			0.004	0.014			0.030				
Mdh–3	A	0.995	0.971	16.38	<0.01*	0.963	0.983	9.54	<0.01	0.980	5.54	0.06	0.83	0.66
	B	0.003	0.024			0.034	0.012			0.020				
	C	0.002	0.005			0.003	0.005			–				
Mdh–4	A	–	–	0.99	0.32	0.004	0.001	2.06	0.36**	–	0.1	0.76	0.35	0.55
	B	0.999	1.000			0.996	0.998			1.000				
	C	0.001	–			–	0.001			–				
Me–1	A	0.002	0.006	5.92	0.05*	0.042	0.036	15.61	<0.01**	–	25.31	<0.01*	36.63	<0.01**
	B	0.996	0.986			0.958	0.945			0.960				
	C	0.002	0.008			–	0.019			0.040				
Mnr–1	A	0.030	0.048	65.84	<0.01*	0.032	0.059	25.58	<0.01*	0.173	70.29	<0.01*	45.05	<0.01*
	B	0.952	0.856			0.930	0.853			0.707				
	C	0.018	0.096			0.038	0.088			0.120				
Mnr–2	A	0.009	0.024	8.17	<0.01*	0.004	0.040	26.95	<0.01*	0.107	45.51	<0.01*	63.24	<0.01*
	B	0.991	0.976			0.996	0.960			0.893				
6Pgd–1	A	0.125	0.177	62.48	<0.01*	0.101	0.128	23.28	<0.01*	0.150	12.17	<0.01*	6.33	0.04*
	B	0.857	0.740			0.867	0.792			0.780				
	C	0.018	0.083			0.032	0.080			0.070				
6Pgd–2	A	–	0.001	15.52	<0.01**	0.049	0.044	11.74	<0.01*	–	15.74	<0.01*	21.73	<0.01*
	B	0.926	0.875			0.883	0.840			0.810				
	C	0.074	0.124			0.068	0.116			0.190				
Pgi	A	–	0.005	11.72	<0.01**	0.011	0.018	1.93	0.38	–	14.04	<0.01*	18.12	<0.01*
	B	0.909	0.869			0.908	0.894			0.790				
	C	0.091	0.126			0.081	0.088			0.210				
Pgm	A	0.989	0.985	0.57	0.45	0.945	0.929	1.69	0.19	0.950	9.8	<0.01*	0.05	0.82
	B	0.011	0.015			0.055	0.071			0.050				
Sdh	A	1.000	1.000	–	–	0.997	1.000	2.18	0.14	1.000	–	–	0.25	0.62
	B	–	–			0.003	–			–				
Skdh–1	A	0.059	0.065	0.3	<0.58	0.095	0.094	0.002	0.96	0.110	3.96	0.05*	0.22	0.64
	B	0.941	0.935			0.905	0.906			0.890				
Skdh–2	A	0.003	0.021	14.07	<0.01*	0.026	0.003	15.72	<0.01	0.010	1.27	0.26	0.93	0.34
	B	0.997	0.979			0.974	0.997			0.990				

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Table 1. (continued)

Locus	Allele	Treatment												
		Seed-tree				Single tree selection				Artificial regeneration				
		F(PR)	F(PS)	χ^2	*P*	F(PR)	F(PS)	χ^2	*P*	F	χ^2	P(1)[a]	χ^2	P(2)[b]
Ugpp-2	A	0.893	0.797	35.4	<0.01*	0.854	0.739	32.14	<0.01*	0.530	99.55	<0.01*	62.21	<0.01*
	B	0.107	0.203			0.146	0.261			0.470				

a χ^2 value and *P* for testing differences between seed-tree (PR) and artificial regeneration.
b χ^2 value and *P* for testing differences between single tree selection (PR) and artificial regeneration.
* Loci showing a significant increase in the frequency of alternate alleles following treatment.
** Loci that gained alleles following treatment.

Table 2. Percentage of polymorphic loci (*P*), mean number of alleles per locus (*A*) and mean number of alleles per polymorphic locus (A_p) for all treatments.

	Treatment				
	Seed-tree		Single tree selection		Artificial regeneration
	Pre	Post	Pre	Post	
P	80.6	87.1	77.4	83.9	74.2
A	2.06	2.29	2.00	2.26	1.90
A_p	2.32	2.48	2.29	2.50	2.22

Table 3. Observed (H_o) and expected (H_e) heterozygosities, and inbreeding values (*F*) for each treatment

	Treatment				
	Seed-tree		Single tree selection		Artificial regeneration
	Pre	Post	Pre	Post	
H_o	0.124	0.101	0.099	0.117	0.197
H_e	0.136	0.104	0.110	0.130	0.177
F	0.088	0.029	0.100	0.100	–0.133

ternate alleles is that it is indicative of a higher level of heterozygosity, since these alleles are likely to form heterozygotes with the more frequent alleles.

Observed (H_o) and expected (H_e) heterozygosities calculated from pooled diploid genotypic frequency data and the fixation index *F* for each stand are presented in Table 3. Observed heterozygosities were lower than the expected for seed-tree and single tree selection stands prior to treatment. Following treatment the seed-tree method resulted in a shift in the observed heterozygosity closer to the expected, whereas the single tree selection method did not result in a change. Consequently, a 3-fold reduction in *F* value was seen due to seed-tree method, while no change was detected in the single tree selection method. Artificial regeneration had considerably higher observed heterozygosity than expected, and consequently a negative *F* value.

A diagrammatic representation of genetic differentiation (F_{ST}) between stands is shown in Figure 1 with the 95% confidence interval for the F_{ST} estimate given in parentheses. The two stands prior to treatment had the same amount genetic differentiation as the two stands after treatment. Similarly, when the seed-tree stand and the single tree selection stand were analyzed

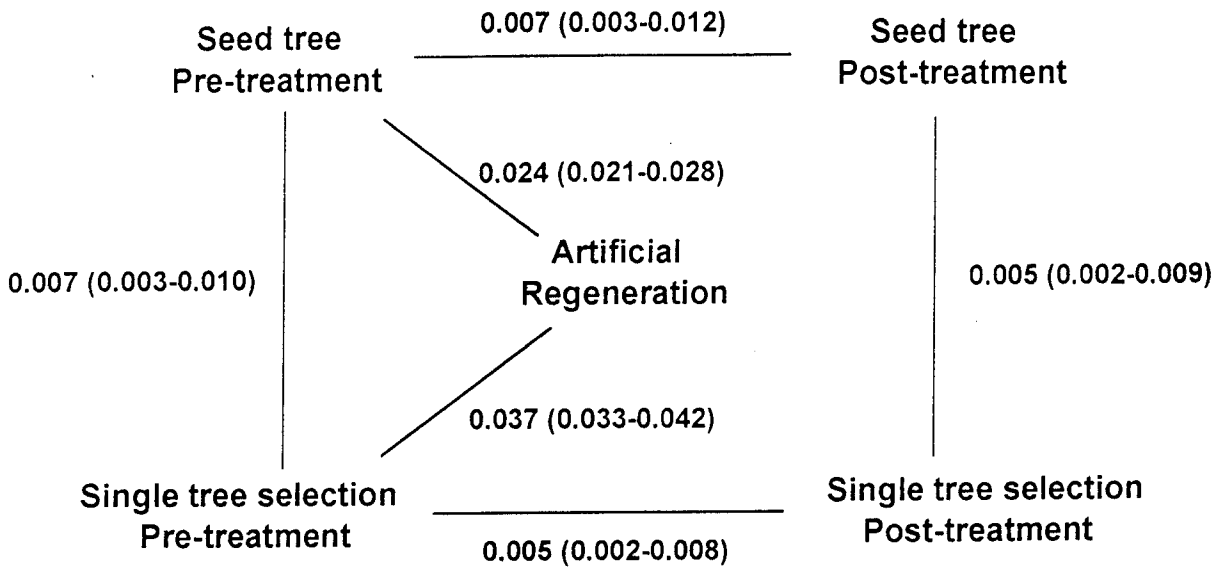

Figure 1. Diagrammatic representation of genetic differentiation (F_{ST}) between stands of shortleaf pine. 95% confidence interval for F_{ST} values given in parentheses.

across time, they showed no change or difference (Figure 1, 95% confidence). Compared to the two pre-treatment stands, artificial regeneration had 3 to 5 fold higher F_{ST} values (0.024 with seed-tree and 0.037 with single tree selection). Artificial regeneration of these stands with seed orchard seed would result in a significant change in the genetic structure of the stands.

When comparing the change in frequency of alternate alleles, it is seen that the increase in frequency of alternate alleles was greatest for artificial regeneration and least for the single-tree selection. H_o, H_e and F values together with allele frequency comparisons confirm that artificial regeneration results in highly heterozygous, outbred regeneration. Regeneration from the seed-tree method showed a reduction in F value, indicative of less inbreeding or consanguineous mating. While the seed-tree method seems to reduce inbreeding, the single tree selection method seems to maintain the existing level of inbreeding. This, of course, may change following additional selection cuts in the single tree selection stand. When the genetic difference between the two stands prior to treatment is considered as base line genetic difference, it can be inferred that the two treatments do not introduce any genetic drift between stands or within stands across time. However, artificial regeneration introduces 2.4 to 3.7% genetic differentiation compared to the previously existing stand. This is a significant change when considering the fact that the total genetic differentiation in shortleaf pine across its natural range is only 9% or lower (RAJA *et al.* 1997; EDWARDS & HAMRICK 1995).

CONCLUSION

Isoenzyme markers were powerful enough to detect changes in genetic diversity and structure in shortleaf pine due to management. The pollen cloud was enriched when the seed-tree and single tree selection regeneration methods were applied to the stands. The reason for such an enrichment may partly be due to the removal of related individuals from the immediate surroundings of the parent tree, and partly due to better access to diverse pollen through the opening up of the canopy. The pollen diversity in the seed orchard was less than that achieved by the two natural regeneration treatments and less than that of the stands prior to treatment, which could be attributed to the limited number of clones in a seed orchard. The seed-tree method increased the frequency of heterozygotes, thereby reducing inbreeding, while the single tree selection method did not alter the level of heterozygotes or inbreeding, and artificial regeneration would result in a highly heterozygous, outbred population. The two natural regeneration systems do not introduce genetic drift, but artificial regeneration seems to introduce a high genetic change compared to the previously existing stands. These results confirm the trend noted by NEALE and ADAMS (1985).

However, we advise some caution in interpreting these results. While the seed-tree stand and artificial regeneration seed sampled reflects genetic changes after the final cut had been applied to the stands, the single tree selection stand sampled the genetic changes after just the first cutting had been applied. In the

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single tree selection system, the regeneration that restocks the stand comes from periodic harvest cuts and hence the genetic composition of the stand's seed after several harvests could possibly be somewhat different from that after the first harvest cut. The results are also limited by the fact that we sampled seed and not the actual regeneration. We recommend an extended study evaluating established regeneration at later stages of all three regeneration systems. The genetic differences we detected were in the seeds, many of which may disappear during seedling establishment owing to natural selection as suggested by MUONA *et al.* (1987) and YAZDANI *et al.* (1985).

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