

THE FOREST SERVICE OPENS A LABORATORY
FOR THE GENETIC ANALYSES OF TREES

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Abstract. In February 1988, the Forest Service established an electrophoresis laboratory to serve the National Forests. The new laboratory will process seeds and tree samples to provide forest personnel with genetic information about relative amounts and geographic patterns of natural variation. Tests at the laboratory will give forest managers the means for evaluating the genetic consequences of stand management practices, and tree improvement workers will have access to new quality control measures for many aspects of their on-going programs.

Additional keywords: Tree improvement, genetic diversity, isozyme.

INTRODUCTION

The genetic quality of seedlings is a major concern in Forest Service planting operations. Accordingly, Forest Service Regions and National Forests have developed and implemented comprehensive tree improvement programs during the last 25 years. Steps have been taken to account for natural adaptation by mapping seed zones, certifying seed collections, and applying seed transfer rules. Emphasis has been given to collecting seed from large numbers of trees, including plus tree selections, within a specific seed zone to maintain high levels of adapted genetic variation. Plus tree selections have been made for some species/seed zones solely to provide a seed source to ensure that adequate genetic variation is maintained in seedlots used in the reforestation program. Most Forest Service plantations are now established with full knowledge of seed origin.

Intensive tree improvement programs have been initiated for several species where the investments can be justified. These programs include: defining breeding zones, selecting plus trees, planting progeny tests to evaluate the genetic worth of the selections, and establishing seed orchards for seed production. The base populations for each breeding zone include large numbers of plus trees to provide high levels of genetic diversity for continuous improvement in future generations. As of October 1, 1987, National Forest personnel have selected 98,348 plus trees, planted 1,205 progeny tests that cover 7,522 acres, and established 185 seed orchards on 2,653 acres (table 1). Advanced generation selection and breeding programs for high priority species are under way in some Regions.

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Table 1.--National Forest System tree improvement program statistics as of October 1, 1987.

Region	Headquarters	Selections		Seed Orchards/ Clone Banks		Progeny Tests	
		Species	Number	Number	Acres	Number	Acres
1	Missoula	6	8,111	11	134	50	519
2	Denver	3	452	--	--	4	6
3	Albuquerque	2	410	--	--	--	--
4	Ogden	2	874	--	--	8	141
5	San Francisco	7	5,722	12	169	49	238
6	Portland	25	75,002	98	725	690	5,371
8	Atlanta	13	3,103	29	1,302	266	892
9	Milwaukee	35	4,674	35	323	138	355
10	Juneau	--	--	--	--	--	--
TOTALS			98,348	185	2,653	1,205	7,522

Rare and endangered species are protected in their native environments, and there is growing awareness of the need to identify the unique genetic characteristics of these species. There is also growing concern and need for information about the genetic effects of various silvicultural systems on the common and wide-spread species. Both needs for information are driven by the realization that particular genes and particular adapted gene complexes are vulnerable to permanent loss; protecting rare species and choosing silvicultural systems to maintain high levels of adapted genetic variation are priority goals in forest genetics programs.

Forest Service policies also mandate applying management strategies to maintain natural diversity; however, Forest personnel have had very limited means to identify and monitor a primary component of natural diversity--genetic variation. Fortunately, there is a timely and efficient way to obtain information about genetic variation. Researchers have developed procedures to identify the products of numerous genes, and those procedures can now be used to evaluate diversity and map geographic patterns of variation. These gene products are also excellent markers for identifying particular trees, their seed and their pollen.

In February 1988, a decision was made to establish an electrophoresis laboratory to produce state-of-the-art genetic information. Two basic objectives will be addressed: to provide analyses in support of wide-ranging and complicated tree improvement programs, and to develop base-line genetic data useful for gene conservation and long-range population management.

The laboratory will perform analyses that identify different forms of enzymes, called isozymes. The enzyme variants provide precise genetic data for a large number of genes within each sample of living tissue. The gene frequencies obtained from those tests provide a timely way to inventory the amount and geographic distribution of genetic variation in native forests. The same gene markers are highly valuable tools for quality control in numerous phases of on-going tree improvement programs.

LOCATION AND ORGANIZATION OF THE LABORATORY

The National Forest Genetics Electrophoresis Laboratory (NFGEL) is located at the Placerville Nursery on the Eldorado National Forest, 45 miles east of Sacramento, California; it is within a mile of the Institute of Forest Genetics (IFG). The new laboratory has offices for the staff, space for working with incoming plant materials, and a large area for conducting laboratory analyses.

The staff will include the lab director, one full-time technician, one part-time technician, and a part-time statistician. The director will be responsible for developing guidelines for collecting samples, submitting samples for analysis, running analyses, interpreting data, and supervising the day-to-day operations. The director will be the primary contact for information, scheduling, and explaining results of specific analyses. The technicians will run the analyses and do general lab work. The part-time statistician will monitor the statistical precision of the laboratory and provide assistance in data analysis. In addition, scientists and technicians from the Institute of Forest Genetics will provide technical assistance. As experience is gained, adjustments will be made to improve statistical precision and the overall efficiency of the lab operation.

National Forest field personnel will be responsible for proposing projects. The laboratory staff will work with the Regions and National Forests to define their special information needs. Interpretive final reports will be delivered to field personnel, and the laboratory staff will be available for consultation. Meetings involving user groups, laboratory staff, and research personnel will be arranged when needed. A steering committee will establish basic policies and resolve conflicts in priorities.

TYPES OF ANALYSES

Forest Service research to adapt electrophoresis techniques to forest trees was initiated in 1962 at the Institute of Forest Genetics (IFG), Pacific Southwest Forest and Range Experiment Station (PSW). Techniques, equipment, and knowledge have improved steadily since then, and electrophoresis has evolved into a powerful tool for analyzing forest species.

This form of electrophoresis refers to a system where enzymes with slightly different molecular structures separate into distinct bands in starch gels when the gels are charged with direct current. Different gene products, the sometimes numerous alleles of specific genes, are "read" as colorfully stained enzyme mobility variants on the gels. For the enzymes analyzed, the rule is one enzyme equals one gene. The procedure has been used to identify genotypes for over 50 genes per tissue sample.

The laboratory is designed to analyze about 250 individual samples per week. Initially, data will be generated for about 20 genes. This number should exceed the statistical requirements for making sound judgments for most applications. Some questions can be answered using 5 to 10 of the most variable genes, thus increasing production to upwards of 500 samples per week.

Eight types of analyses have been identified as examples of work the laboratory will perform:

1. Map patterns of genetic variation. Isozyme analysis of seeds from trees scattered over wide areas can provide valuable information about geographic patterns of genetic variation. While some species have very little geographic variation, and therefore have broad seed zones, other species change genetically over relatively short distances and therefore require much smaller zones to ensure adaptability. Isozyme data can also be used to refine seed zone and breeding zone boundaries, and to assist with the allocation of plus trees to seed orchards.

2. Evaluate management effects on genetic diversity. Questions are frequently asked about the effects of various silvicultural systems on genetic diversity. Specific analyses will be made to determine if the regeneration is more variable, less variable, or about the same as that of the preceding generation. This type of analysis usually begins with a genetic description of the original stands, followed by describing variation in the seedlings and young trees present in the new stands.

3. a) Check compliance of cone collectors. The Forest Service generally collects over 50,000 pounds of seed annually. Over half of this seed is collected by contractors and/or Forest Service employees from designated stands within seed zones. By analyzing seeds from single cones collected from several trees growing within the designated stands, the laboratory staff can estimate the gene frequencies of these trees and compare this to the gene frequencies present in the final bulked seed collection. Likewise, if single-tree collections were requested, tests could verify that pure collections were obtained from the appropriate trees.

b) Check commercial seedlots. In the past 10 year period, the Forest Service purchased an average of 4,100 pounds of seed annually from commercial sources. The origin of these commercial seedlots could be verified by collecting new seed samples from the appropriate stands and then testing for close genetic relationships between the commercial and test seedlots.

4. Identify ramets, families, provenances, and seedlots. Mistakes in labeling occur in field tests, seed orchards, and seed banks despite the best precautions and controls geneticists and nursery managers implement to prevent them. Isozyme analysis of suspicious materials will often help reveal identities and raise the consciousness of workers to avoid similar mistakes in the future.

5. Verify specific crosses. Isozymes are the only readily available means for checking the identity of controlled crosses. As we move into advanced generation breeding programs, quality control for pollination and verification of parentage is essential.

6. a) Estimate pollen dispersal and seed orchard contamination. Pollen distribution and orchard contamination can be estimated using genetic markers from pollen sources within seed orchards and from pollen sources within surrounding stands. This knowledge will help managers to adjust procedures to achieve their seed orchard objectives.

b) Check effectiveness of supplemental mass pollination techniques. In order to make reasonable estimates of genetic gain from mass pollinated orchards, we need to know that the desired pollen fertilizes the orchard. Isozyme markers will also be used to develop cost-effective supplemental mass pollination techniques.

7. Quantify the rates of inbreeding and outcrossing. Knowledge of the breeding system is critical to the management of stands and seed orchards. In some cases it may not be wise to depend on natural regeneration because of a stand's narrow genetic base and tendency for substantial inbreeding. Knowing the proportions of selfed and outcrossed seeds produced by clones in seed orchards will provide the basis for modifying seed orchard management strategies.

8. Monitor genetic diversity in recurrent mass selection programs. The genetic make-up of plantations changes in response to natural selection and in response to the chance elimination of trees with specific genetic traits. Monitoring genetic change within a stand over a rotation will lead to understanding relationships between genetic factors and stand development. Monitoring variation over several rotations will lead to understanding evolutionary processes in forest populations. Isozymes can also be used to select individual trees from a population of selected trees to ensure that genetic diversity is maximized in the resulting plantations.

BENEFITS

The Forest Service will achieve benefits in two broad areas: 1) increased precision and application of new quality controls in Regional tree improvement programs, and 2) production of extensive new base-line information on genetic diversity for a variety of forest tree species that can be used to validate management strategies.

Including the best selections in breeding programs will increase the potential wood production on National Forest System lands. Conversely, if selection is compromised, the potential will not be achieved. Errors in seed and/or seedling labeling and handling, from seed collection through seedling outplanting in seed orchards or progeny tests, are inevitable. Using isozyme analyses to validate specific crosses/selections/progenies will help geneticists confirm that they are indeed selecting the specific genetic material that is desired. This is especially significant in forest genetics programs because of the extended time periods involved in evaluating performance of progenies derived from breeding programs. Eliminating parents from advanced generation programs is very costly as the progenies involving these parents must be rogued, and, more important, the genetic base in the program may be reduced. This may require adding new material to the breeding population, and this most likely will reduce the genetic gain in the short term. It is critical to base selections on all available information to reduce the probability of having to make changes in later generations. Isozyme data is another tool that will help geneticists develop confidence in their selections, and thereby achieve potential wood production goals sooner.

Gene pool conservation and genetic diversity have been topics of debate over the past few years. Basically, what effect does our timber management program have on the gene pool? On genetic diversity? Very little substantial information is

available to answer these questions. The Forest Service, managing a wide-range of diverse habitats, has an opportunity to provide international leadership on the effects of management on genetic diversity and the gene pool, as both managed and relatively pristine populations of forest trees are available for study. Monitoring allele frequencies over time on sites receiving different silvicultural treatments will provide the necessary data. With this data in hand we can support our management strategies in respect to effects on the gene pool, and, if necessary, we can modify our treatments if data shows there are in fact adverse impacts on genetic diversity.

SUMMARY

The establishment of an electrophoresis laboratory will benefit the Regions' tree improvement programs, seed procurement programs, and the overall timber management program. The lab is located in existing facilities at the Placerville Nursery, and will be operated by a professional and the equivalent of two technicians. The Forest Service is implementing state-of-the-art technology developed by Forest Service Research at PSW, and will continue to take advantage of the latest information as it is provided by the scientists at the Station.

Data will be used to validate specific genetic materials adding confidence to our advanced generation tree improvement programs, and to provide information on the effects of timber management on genetic diversity and the gene pool.