

The Forest Service Laboratory for the Genetic Analyses of Trees

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In February 1988, the USDA Forest Service established an electrophoresis laboratory at Placerville, CA, to serve the National Forest System. 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. Tree Planters' Notes 40(4):25-29; 1989.

The genetic quality of seedlings is of 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 natu-

ral adaptation by mapping seed zones, certifying seed collections, and applying seed transfer rules. Emphasis has been given to collecting seeds 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. 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, 1988, National Forest System personnel have selected 101,406 plus trees, planted 1,319 progeny

tests that cover 8,508 acres, and established 204 seed orchards on 2,861 acres (table 1). Advanced generation selection and breeding programs for high priority species are under way in some regions.

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 on the effects of various silvicultural systems on the genetics of common and widespread species. Both needs for information are driven by the realization that certain genes and gene complexes are vulnerable to permanent loss. Protecting rare species and choosing silvicultural systems to maintain high levels of adapted genetic variation are priorities in forest genetics programs.

Forest Service policies also mandate applying management strategies to maintain natural diversity; however, 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

Adapted from a paper presented at the Northeastern Forest Tree Improvement Conference, July 7, 1988, at Pennsylvania State University, University Park, PA.

Table 1—National Forest System tree improvement program statistics (October 1, 1988)

Region	Headquarters	Selections		Seed orchards/ clone banks		Progeny tests	
		Species	No.	No.	Acres	No.	Acres
1	Missoula	6	8,192	13	141	52	534
2	Denver	3	452	—	—	4	6
3	Albuquerque	2	437	—	—	—	—
4	Ogden	2	874	—	—	8	141
5	San Francisco	8	7,663	12	192	49	266
6	Portland	26	75,629	117	903	767	6,219
8	Atlanta	14	3,422	27	1,302	301	987
9	Milwaukee	35	4,737	35	323	138	355
10	Juneau	—	—	—	—	—	—
Totals			101,406	204	2,861	1,319	8,508

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.
- To develop base line genetic data useful for gene conservation and long-range population management.

Electrophoresis

In electrophoresis, a buffered solution of ground reproductive or vegetative tissue is absorbed by paper wicks that are inserted into a starch gel. When an electric current is applied to this gel, different molecules in the solution will migrate down the gel for various distances, depending on their charge and molecular weight. Thus, enzymes with slightly different molecular structures separate into distinct bands in starch gels when these gels are charged with direct current. Each gel can be layered into about six slices and each slice differentially stained. The products of different genes, as well as the sometimes-numerous alleles of a specific gene, are "read" as colorfully stained bands 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 will perform analyses that identify the different forms (isoenzymes, or isozymes) of various enzymes. 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. Unique enzyme variants can be highly valuable markers 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, CA; it is within a mile of the USDA Forest Service 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.

National Forest System 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.

Analyses of Electrophoresis Data

Forest Service research to adapt electrophoresis techniques to forest trees was initiated in 1962 at the Institute of Forest Genetics. Techniques, equipment, and knowledge have improved steadily since then, and electrophoresis has evolved into a powerful tool for analyzing forest species. The Forest Service laboratory will conduct analyses to resolve problems and answer questions in the following areas:

Map patterns of genetic variation.

Isozyme analyses 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.

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.

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 singletree collections were requested, tests could verify that pure collections were obtained from the appropriate trees.

Check commercial seedlots.

In the past 10 years, 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.

Identify ramets, families, provenances, and seedlots.

Mistakes in labeling occur in seed banks, nursery beds, field tests, and seed orchards 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.

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.

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.

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.

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.

Monitor genetic diversity in recurrent mass selection programs. The genetic make-up of plantations changes in response to natural selection and 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. Isozyme analysis 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:

- * Increased precision and application of new quality controls in regional tree improvement programs.
- Production of extensive new baseline 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 importantly, 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 are 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 these 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 show there are in fact adverse impacts on genetic diversity.

Summary

The establishment of an electrophoresis laboratory will benefit regional tree improvement programs, seed procure-

ment programs, and the overall timber management program. The laboratory is located in existing facilities at the Placerville Nursery and will be operated by a professional staff. The Forest Service is implementing state-of-the-art technology developed by Forest Service researchers at the Pacific Southwest Forest and Range Experiment Station and will continue to take advantage of the latest information as it is provided by the scientists.

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.

Further Reading

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