

PROVENANCE RESEARCH: INVESTIGATION OF GENETIC DIVERSITY ASSOCIATED WITH GEOGRAPHY

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4. Provenance research: investigation of genetic diversity associated with geography

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Summary

Provenance in forestry refers to the population of trees growing at a particular place of origin. Provenance research defines the genetic and environmental components of phenotypic variation associated with geographic source. Information on provenance is important in assuring sources of seed to give well-adapted, productive trees and in directing breeding of interracial and interspecific hybrids toward adaptation to particular localities. Concepts of the species, of variation within species, of continuity in this variation, and of relation of variation to factors of the environment have developed over the past century. Studies of provenance should start with a com-

prehensive summary of information on genetic and environmental variability within the range of the species. Objectives and procedures of research should be well defined. Many questions must be considered in designing and executing a sample of many provenances to accomplish objectives. Biosystematic studies utilizing several disciplines should be most efficient to define patterns of variation and natural biotic units having similar form and function. Seed source studies will demonstrate adaptability, growth, and yield. They may also serve as seed sources. Seed source studies in semi-arid lands are faced with unique problems.

Chapter 4

The choice of seed sources is one of the main factors affecting the establishment and productivity of plantations of forest trees. In the present practice of silviculture, provenance research provides a sound basis for the selection of seed sources. Anyone concerned with afforestation and reforestation should develop a program of provenance research to assist in the selection of seed sources. Such valuable work has been done in the past. Extensions of this and new research are vitally needed on an international scale because without it future investments in afforestation and reforestation will not return their maximum revenue. Provenance research should be given highest priority at the outset of any program of forest tree improvement.

Provenance research means many things to many men. It is, therefore, useful to begin by defining some of the terms in common use, outline the scope of the subject, and mention its general importance. The

concepts of genetic diversity within species emerging over the past 140 years can also be profitably reviewed. The species, variation within species, and the nature of this variation, whether it be continuous or discontinuous, all need to be considered. Finally, consideration must be given as to how to study the great diversity within species. Through the modern biosystematic approach patterns of variation can be defined. Through the familiar seed source study the most productive provenances can be found. By combining both approaches the goals of provenance research will be most efficiently attained.

MEANING OF PROVENANCE

Terms referring to that part of the genetic diversity within species which is associated with geography must be defined at the outset to provide a common vocabulary. "Provenience" and "provenance" are two words having the same meaning. Both refer to origin or source. They are fully interchangeable, and for consistency only the word provenance is used here.

Provenance in forestry refers to the particular place where trees are growing or the place of origin of seeds or trees. Provenance may refer either to native or to planted trees growing at that place, but its common

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use is in reference to native trees. By definition and some usage provenance could also refer to an individual parent tree. However, provenance should be restricted to refer to the population of trees growing at a place and not to an individual tree. Hence, "selecting the proper provenance" means selecting the proper locality.

Use of the word provenance in reference to a region is improper. This use stems from provenance experiments. In such experiments trees from one local source might differ from all others. In some cases reference has been made to the broad region represented by one source as a provenance. More refined research undoubtedly would show this region to be made up of many provenances.

Thus, provenance has a biological meaning roughly equivalent to a local population or deme. A provenance will be some part of a race, ecotype, cline, subspecies or variety.¹

Provenance research aims at defining the genetic and environmental components of phenotypic variability between trees from different geographic origins.

The broad scope of provenance research involves all studies above the level of the individual and below the level of the species. Langlet (1962) regards the investigation of provenances as "...the study of ecological variability within species, the relationship between this variability and the influence of environment, and the reactions of different populations to transfer to an environment foreign to them..." The scope of provenance research in its broadest concept should include:

1. studies of inherent adaptive variation related to "ecological variability within species";
2. studies of the inherent nonadaptive differences that might result from isolation or other factors.

This broadened concept coincides with the concept of intraspecific studies in biosystematics or experimental taxonomy. The classical seed source studies of North America or the synonymous provenance experiments of Europe (Edwards, 1956) are a part of provenance research. They only study the reactions of different provenances growing in the same places.

Information from provenance research has great practical use in the improvement of forest stands. The most obvious and most important use at present is to assure sources of seed that give well-adapted, productive trees in reforestation and afforestation. Provenance research may take a long time to find the best seed source for any particular area, but it should define an acceptable seed source after one or two generations

of testing. If juvenile characters can be used to evaluate performance, then the time may be shortened.

Provenance research also has importance for the planned breeding of hybrids between species. As was demonstrated in Chapter 3, crossing species is not enough. One must direct breeding to produce a hybrid combination of provenances having the highest adaptation and productivity. Duffield (1954) states, "Performance of hybrids is greatly influenced by the racial origin of the parents... The species hybridizer cannot escape the provenance problem."

Another important aspect of provenance research is the information provided for breeding hybrids within species. Hybrids combining different provenances often combine desirable characters of different races and may result in hybrid vigor for many characters (Clausen, Keck, and Heisey, 1948; Wettstein, 1958). Such hybrids may be adapted to a wider range of environmental conditions than were the parents. With knowledge of inherent geographic diversity the forest geneticist can choose the proper seed sources and direct hybridization of races within species to his advantage.

CONCEPTS OF DIVERSITY WITHIN SPECIES

Concepts of variation within species developed as species of trees came to be understood from a taxonomic and a biological viewpoint. Long after a firm concept of a species had emerged, the variation within species became apparent to both taxonomists and foresters. Foresters preceded other scientists in recognizing this variability, the continuity of this variation pattern, and its relation to environment (Langlet, 1962). They recognized that environment has two effects: a direct effect on the expression or plasticity of the phenotype; and an indirect effect in guiding evolution of population. Their seed source studies, followed by modern biosystematic² investigations, are beginning to resolve the patterns of broad variation and local differentiation in forest tree species.

The species concept

The present concept of species has emerged over several thousand years. Earliest taxonomists described similar individuals and gave them a name and this culminated in the binomial system of nomenclature credited to von Linné. Names must be assigned on relatively nonvariable characters; hence, highly heritable characters considered to be conservative have been used to distinguish species. This has caused taxonomists to search for and concentrate on stable characters. Those characters lacking uniformity and showing diversity have had less attention.

Today the concept of a species as a group of uniform,

¹ For original definitions of these taxonomic terms and interpretation of their use, consult: Turesson (1922, 1925, 1930); Huxley (1938); Clausen, Keck, and Heisey (1940, 1948); Stebbins (1950); Lawrence (1951); Heslop-Harrison (1953); Snyder (1959) or some modern text on plant taxonomy.

² Biosystematics in the unrestricted sense is a phase of botanical research that endeavors, by study of living populations, to delimit the natural biotic units. (See Lawrence (1951) and later in this chapter.)

immutable individuals has been discarded in favor of the neo-Darwinian concept. Now the science of genetics has established the genic basis of isolation between species. It also provides a means to account for the heritable variation observed within species.

Variation within species

Differences within species were apparent to taxonomists who collected them in various locations, and to foresters concerned with moving seed from place to place early in the nineteenth century. De Vilmorin was among the first to make comparative plantings of seed from several sources on a common site at Les Barres, France, from 1823 to 1850. Subsequent seed source studies have become more sophisticated in design and broader in scope. Much of this work has been summarized by Schreiner (1937), Kalela (1937), Langlet (1938), Schütt (1958), and Squillace and Bingham (1958).

Many studies of variation within species made over the past 140 years have led to these general conclusions and their corollaries:

1. A diverse environment throughout the range of the species leads to a genetically variable species. Wide-spread species tend to be more variable than restricted species.
2. Patterns of inherent variation parallel patterns of environmental variation. Discontinuities in patterns of inherent variation are related to breaks in the distribution of a species or rapid changes in environmental factors.
3. Races of a species growing in different climatic regions may differ in inherent adaptation to environmental factors. In one region a certain factor of the environment may be critical; in another region this factor may be less important than some other critical factor.
4. Sympatric species will be similar but not identical in inherent adaptations to the same environment. Limiting factors generally are not always the same for cohabiting species.
5. Two or three successive seed source trials will be needed to determine an optimum source. Most species and environments are too variable to be analyzed completely in one experiment.
6. Seed source studies of native species undisturbed by man generally show the local seed source to be the best adapted but not necessarily the most productive. Exotic populations do not equal local populations in adaptation to the unique combination of factors of the local environment.
7. The local seed source is safest if little is known about variation in a native species.
8. Performance will be unpredictable for species grown for a long time under cultivation or disturbance by man or for species transferred to radically different environments, as in the case of species of *Eucalyptus* and *Cedrus*.

Continuity of variation

The continuity of variation in certain characters has been convincingly demonstrated for a few species. European research foresters were pioneers in this work (Langlet, 1959). Cieslar and Engler were the first to demonstrate continuous change within species. Wibeck, Schotte, and Langlet related this inherent variation to continuously varying factors of the environment, such as temperature and growing period.

Regression techniques have commonly been used to demonstrate continuity of variation but their use has a certain hidden danger. Through regressions one can bridge discontinuities or fail to recognize individual populations which deviate significantly from the regression. Care must be taken in the use of this important tool of analysis and in interpreting results.

Langlet (1962), considered by many to be the foremost proponent of continuous variability, himself points out: "Of course, not all ecological variability is geographically continuous. There may be more or less definite limits and discontinuities. These may result, for example, from different kinds of genetical or environmental isolation, from abrupt changes in environment, a sharp limit to a high plateau, sharp boundaries between edaphic conditions, scarcity of suitable ecological niches, or simply haphazard geographical distribution."

Turesson (1922) explained his original observations of discontinuous variation patterns by the ecotype concept. Many authors have used this concept in describing variation in a forest tree species.³ A critical review of past studies shows that an investigator must use caution in describing ecotypes. The fact that two populations differ significantly does not justify calling them ecotypes or assigning them subspecific names. One must insure that observed discontinuities in a species are not an artifact of discontinuous sampling (Langlet, 1959).

An investigator studying patterns of variation must avoid a biased approach in studying, interpreting, or reporting the patterns. Clines and ecotypes, like regressions and analyses of variance, are not mutually exclusive concepts. Continuous versus discontinuous variation can only be resolved by thorough, well-designed studies.

If major discontinuities or very steep gradients are detected from competent investigation, judicious use of names to define subspecific taxa seems to be appropriate. Scientific names, like other words, must have understandable meanings. Names should only be applied to discrete segments of a species after comprehensive studies of patterns of variation throughout its range. A good example is the thorough study and designation of subspecies of *Pinus contorta* Dougl. by Critchfield (1957).

³ Wright 1944a, 1944b; Pauley and Perry, 1954; Mc Millan, 1956; Kriebel, 1957; Wright and Baldwin, 1957; Habeck, 1958; Vaartaja, 1959.

STUDYING GENETIC DIVERSITY WITHIN SPECIES

A complete provenance study has these basic steps:

1. summary of available information on variability within and between populations;
2. decision concerning the objectives and procedures in the light of known and expected patterns of variation;
3. design and collection of a sample of many provenances to accomplish the objectives;
4. biosystematic investigations of provenances to describe patterns of variation;
5. establishment of seed source tests on representative sites for a few of the most promising or typical provenances;
6. integration of the results of biosystematic and seed source studies to define regions most likely to produce the most suitable seed.

A combined approach using both biosystematic and seed source studies should be most efficient (Callahan, 1961). Biosystematic investigations can encompass many more provenances than can seed source studies. Yet seed source studies provide the actually growing proof of the suitability of particular provenances. Extrapolation from the results of seed source tests will be most efficient if biosystematic investigations have been completed. Neither biosystematic nor seed source studies alone will provide the information needed for designating the optimum seed source.

One should not expect all the required information from the first provenance studies. Past research suggests that the ideal provenance test cannot be designed and executed without information on variation patterns from preliminary tests. Even an ideal test may have to be followed by other tests to find the best or optimum sources of seed.

Summary of present knowledge

A critical review of the information available should provide considerable information on geographic variation within a species. Taxonomic literature may provide the most leads. Descriptions of subspecific taxa may show the range of variation from place to place, and they may indicate locations of discontinuities in the variation pattern. Comparisons of samples from many herbaria may disclose previously unreported variation.

Existing seed source studies, if available, will provide the best leads. They may show broad or local patterns of variation in many characters. Critical environmental factors responsible for dominant variation patterns in the species may be apparent. Comparisons of results from different studies or plantings may reveal interactions between seed sources and the environments of planting sites. The most important contribution of such studies may be indications of the heritability of individual characters and the plasticity of the phenotypes.

Surveys of information from other scientific disciplines may show patterns of variation in size, form, or function within the species. Plant physiologists, ecologists, and silviculturists may have shown the critical environmental factors to which the species is inherently adapted. They may also have compared trees from different provenances with respect to growth period, assimilation rates, foliage pigments, and many other characters. Studies of comparative anatomy, morphology, and biochemistry may show patterns of variation. Morphology of organs, internal anatomy, biochemistry of essential oils and other compounds, and many other characters, may vary through the range of the species. Climatologists usually have information on patterns of variation in temperature and precipitation during critical growth phases. They may also know about the occurrence of other critical climatic factors, such as early or late frosts, deep or wet snows, sleet, sudden temperature changes, and unusual winds. Soil scientists and geologists may provide basic information on the nature of parent rocks and of derived soils throughout the distribution of the species. Starting an investigation without first bringing together all these sources of information would be most inefficient.

Planning the study

Surveying the existing literature will bring questions to mind and will point to the objects of the research to be undertaken. The objects of the study will in turn determine the balance between the two approaches. Biosystematic studies are comparatively inexpensive, and produce the most information on variation per unit of investment. On the other hand, while seed source studies are expensive, they provide proof of adaptability and productivity. If little or nothing is known about the species to be studied, biosystematic investigation should be paramount. If optimum seed sources have been suggested by previous studies, seed source studies concentrating on growing trees from certain regions in a crop experiment (Edwards, 1956) may be most important. Any comprehensive investigation will use both approaches, giving one more or less weight than the other.

Sampling the species

The next step is to sample the species to provide the material for study. Many questions now arise: Where to sample? Which trees to sample? What and how many specimens to take? When to sample? Who is to sample? How to document the samples?

Locations. The investigator must determine the extent of coverage desired, how many locations can be sampled, and what particular locations should be sampled. Broad coverage of the entire range of the species will be required if little or nothing is known about patterns of variation. If the existence of a region producing well-adapted, productive trees is suspected or known, intensive coverage of major and minor environmental

COLLECTION DATA

SPECIES:	Scientific name and author _____		
	Common name _____		
ORIGIN:	Country _____	State or province _____	County _____
	Latitude _____	Longitude _____	Elevation _____ feet or meters
	Detailed location (section, township, range, meridian; direction and distance to town or other landmark) _____		
	Slope direction _____	Slope steepness _____	%
	Forest type _____	SAF type number _____	Site index _____
	Soil pH (basic, neutral, acidic) _____		
	Soil moisture-site condition (dry, moist, wet) _____		
	Other tree species in stand _____		
COLLECTION:	Date of collection _____		
	Number trees in collection _____	Age _____	
	Average height _____	Average diameter _____	
<i>Cross out word NOT applicable:</i>			
	Collected from: (standing trees) (felled trees) (rodent caches)		
	Trees are in: (plantations) (natural stands)		
	Trees are in: (open) (thin stands) (dense stands)		
	Shedding of seed or fruit: (not started) (starting) (underway) (complete)		
	Method of extraction: (air dried) (kiln dried at _____°C.)		
	(crown form, branching, vigor, disease, insect damage, etc.)		
REMARKS:	_____		

Date _____	Signatures _____	Collector	
Address _____	_____	Dealer	
_____	_____	Forester	

FIGURE 15 - Suggested form for documenting seed collections. (Prepared by the Committee on Forest Tree Improvement. Society of American Foresters)

variables plus a few samples representative of other regions might be in order. For biosystematic studies, thousands of trees from hundreds of locations might be sampled. For seed source studies, the size of the sample might be limited to hundreds of trees from tens of locations.

Deciding which specific locations to sample within the desired coverage is most difficult. Primary sampling should be done parallel to major environmental gradients within taxa. For example, samples could be taken along specified degrees of latitude, on both sides of every major mountain range, and at specified intervals of elevation, such as 300 to 500 meters. Ideally, at least 3 to 5 samples should be taken along each major gradient in the environment. Secondary sampling should complete the picture by filling in samples along mountain chains and by bringing in unique populations. These latter populations might be outlying stands or suspected plus or minus stands.

Kind of trees. After deciding which locations to sample, the investigator must decide which trees to sample. A sample representing all classes of trees in proportion to their frequency in the stand would show the range of natural variability in the population. This type of collection is neither practical nor desirable. Probably a sample of the dominant and codominant trees in a stand, avoiding immediate neighbors, would be most satisfactory. However, this too may be impractical if co-operators have to be relied upon to make the collections. Seed should never be collected from isolated seed trees because of the high chance of self-pollination.

Preserving the identity of individual trees through the collection stage is commendable. This permits biosystematic study of genetic variation within and between populations. An investigator will not, however, be able to retain the identity of many individual trees when establishing seed source studies in the field.

Number of trees. Ideally, the number of trees to be sampled at any locality should fluctuate with the phenotypic variation between trees at that locality. More specifically it should fluctuate with the variation of the most important or most variable trait. If the trees to be sampled were relatively homogenous, perhaps 5 to 10 individuals would suffice. If the sample trees were heterogeneous, 25 to 50 or more might be appropriate. Since the investigator usually does not have an estimate of the variance between trees at each location, common practice is to collect from 10 to 25 individuals at each location. In no case should the sample to represent an area be limited to only one or a few trees. As far as possible every tree should be equally represented in each seed lot.

Timing. Collections should be organized at least two months before seed ripening and should be made only in years of abundant seed production. This gives the collector an opportunity to search for stands and trees to meet specified requirements. Collections in years of

meager seed production will result in collection from atypical trees, selection of undesirable alternate locations, and high costs of collection. General or regional failures in seed production doubtless will occur. These require repeated collections in key regions or key localities in subsequent years. In this event, long-lived seed can be stored in refrigerators without serious loss in viability. If this is done, crop year effects may have to be considered in subsequent nursery tests (Callaham and Hasel, 1961).

Documentation. The investigator should specify the kind and amount of information required to describe and document the origin of his material (Rohmeder, 1962). Forms should be provided for entering the required information. Detailed descriptions will be needed of the sample trees and locations (Figure 15). A map showing the location of each collection should be requested from the collector.

Collections by co-operators. To effect an economic saving at the outset of a study by making collections through co-operators may be shortsighted. Provenance studies are expensive, and they have both long-term and large economic implications. The best and recommended procedure is for the investigator to go to the stands and make the collections himself. Often this is impossible; hence the next best procedure is for him to organize the work on the ground by personal visits to enlist and instruct co-operators. If this is not possible, someone on the ground may arrange for these collections in the same way.

Certainly, Fielding's (1962) suggestion to organize collection services in remote areas is worthy of implementation. If collection services cannot be provided in remote areas, international co-operation in organizing expeditions for seed collection should be arranged.

Only as a last resort should one make collections by "mail order." If this is necessary, extra precautions must be taken to insure that collectors are properly motivated and instructed to do the required job. Investigators forced to collect via the mails may feel that "beggars cannot be choosers." They may obtain far more than expected if they:

1. give adequate statements of their objectives and justification for their requests;
2. give both general and specific requirements with tolerance for adjustment in collections;
3. provide data sheets showing the kind of information and details needed;
4. provide funds for collection and shipment should costs be prohibitive for collectors;
5. acknowledge any and all assistance received promptly and profusely.

Executing collections. Execution of the sampling design calls for close attention to all the foregoing points. Arrangements for field collections must be

completed well ahead of seed ripening. Collectors must be located, instructed, trained, and equipped as required. Particular attention must be given to quarantine restrictions on the movement of plant materials. The principal investigator should be available to answer questions from collectors during the collection period. He will have to consider and approve alternative procedures or locations as necessary. Processing of collections must proceed rapidly after they are received. At every step the identification of all specimens as to their source must be preserved. These materials then will provide the subjects for future study in biosystematic or seed source investigations.

Biosystematic studies

Biosystematics (or genecology following Turesson) is the comprehensive study of variation within and between species. According to Lawrence (1951, p. 169): "Biosystematics, in the unrestricted sense, is a phase of botanical research that endeavors, by study of living populations, to delimit the natural biotic units... This necessitates use of data from the fields of ecology, genetics, cytology, morphology, phytogeography, and physiology, particularly as observed from plants grown under artificial and natural conditions of environment."

Sylvén (1916), one of the first foresters to conduct a biosystematic study, compared the morphology of *Pinus sylvestris* L. from 59 districts in northern and central Sweden. Weidman (1939) was probably the first to compare the morphology of specimens from trees growing in many native stands with the morphology of their progeny growing in a seed source study. By this technique he was able to estimate the genetic component of variation and the environmental modification in each character. A comprehensive biosystematic study should include such morphological comparisons of native and cultivated trees, but it must also include cytological, physiological, and ecological comparison.

Comparable specimens from many sources should be studied for similarities and dissimilarities in form and structure.⁴ Variability should be investigated in leaves, fruits, wood, bark, and other tissues. Herbarium samples might be used for such studies, but they have a basic failing of not being comparable collections. All characters should be studied with respect to the variation between native trees and between trees growing in common arboretum or seed source tests. These com-

parisons should give some idea of the heritability of characters and plasticity of phenotypes.

Seedlings representing trees from different provenances should be grown in a variety of uniform or, preferably, controlled environments.⁵ This will permit an assessment of heritability of characters and the interaction between genotypes and environments. Some characters which might be studied profitably are: rates of germination and subsequent growth, time of bud break, time of bud set, length of the growing season, susceptibility to early and late frosts and to damage by pests, needle color, and nutrient requirements for growth.

Particular emphasis should be given to studying the nature of photoperiodic and thermoperiodic controls of growth and of phototropic responses for different provenances.

Results from these and other lines of research, such as comparative cytology and biochemistry, should be integrated. Natural biotic units having similar form and function should be described. Multiple regression analyses will be useful in such integrations. A discriminate function analysis may help to define variants and to determine whether patterns are continuous or discontinuous (Hopp, 1943; Whitehead, 1954; Mergen and Furnival, 1960). If analyses of variance in any study show significant differences between provenances, then multiple range tests (Duncan, 1955; Kramer, 1956) can be used to compare means in order to determine which provenances differ significantly. Analyses should be made of the interrelationship between characters measured on each tree. Variation in these characters also should be related to variation in the dominant environmental factors found at the sources (Langlet, 1961; Squillace and Silen, 1962).

The most difficult problem of biosystematic investigations will be to detect and determine the significance of subtle differences between adjacent populations. The problem is to determine whether significant genetic differences exist between populations on adjacent north and south slopes, between populations at the tops of dry ridges and the bottoms of adjacent moist valleys. A few studies suggest that genetic differences exist between adjacent stands (Weidemann, 1930; Squillace and Bingham, 1958; Callaham and Hasel, 1961). Soil factors may be very important in this local variation, and few studies have been made of genetic adaptation to soil differences.⁶ Present knowledge about very local variation would be greater if past studies had retained the identity of individual trees. Differences between trees growing on adjacent sources will be exposed only by critical experimentation. Only when these problems are solved can the best source of seed be designated. When experimentation of this kind has progressed much further, present ideas of the local population may have to be radically revised.

⁴ Space does not permit an exhaustive summary of past studies. Publications on some typical studies in the last decade are by Bouvarel (1954), Nylinder and Hägglund (1954), Carlisle (1955), Priehäusser (1956), Critchfield (1957), Wettstein (1958) and Thorbjørnsen (1961).

⁵ Publications on some typical studies in the last decade are by McMillan (1953), Pauley (1954), Wassink and Wiersma (1955), Schmidt (1957), Downs and Piringer (1958), Leibundgut (1958), Vaartaja (1959), Nienstaedt and Olson (1961), Callaham (1962), Irgens-Møller (1962), Perry (1962), and Wright and Bull (1963).

⁶ de Philippis (1937); Vinogradov (1949); Cyprus Forestry Department (1954); McMillan (1956); Fukarek (1958); Habeck (1958); Canada Forestry Branch (1960).

Seed source studies

Seed source studies are a part of the biosystematic investigation of variation. An investigator plants seeds in one or more nurseries to grow seedlings for planting at a number of field locations. Survival, adaptability, and productivity of seedlings and trees are assessed to find the best source of seed for a particular area.⁷ Such studies are common and have considerable influence on decisions about provenance. Due consideration must, therefore, be given to their design and execution.⁸

Planning and execution of all phases of seed source studies could well follow the lengthy descriptions by Johnsson (1952), Southern Forest Tree Improvement Committee (1951, 1952a, 1952b), and Edwards (1956). Wright (1960) gives some helpful techniques for handling and assessing seeds from many sources. Cultural practices generally will be those already prescribed for the species under study.

The investigator planning the nursery phases should consider the following:

1. Experimental designs incorporating replication and randomization should equalize environmental conditions in the nursery to show early genetic differences and minimize the extension of nursery influences into plantations.
2. Differences in the quality and condition of nursery stock from one nursery to another persist in later forest assessments.
3. Environmental variation related to seed size, sowing time, speed of germination and seedling density should be measured.
4. Frequent observations are needed to detect differences between provenances in such variables as: cotyledon number; amount and duration of growth; presence of dormant buds at the end of the growing season; number, length, thickness, and color of needles; number, length, and angle of insertion of branches; susceptibility to temperature extremes and pests; length and conformation of root systems; fresh and dry weight; and other characters.

The investigator planning the plantation phases should realize that plantation establishment is the most critical and costly phase of provenance research. To obtain the most information with a minimum invest-

ment of labor, money, land, and time, he should consider the following:

1. Sites should be located in reference to gradients in major environmental variables and not in a haphazard or fortuitous manner. Ideally each location should have soil descriptions and long-term climatic records. Wherever possible, plantations should be located on sites having certain combinations of environmental factors representative of a large area of potentially plantable land (Jackson, 1962).
2. The number of plantations required will be dictated by the gradients mentioned above. To protect the study from plantation failure, 20 to 30 percent more plantations must be established than are actually needed for the experiment.
3. Experimental designs of plantings using complete or incomplete blocks of randomized plots seem to be the most suitable for seed source studies. Use many blocks with plots containing a few, 3 to 20, noncontiguous trees for short-term experiments to screen many relatively unknown sources for general adaptability and early growth assessments. Use fewer blocks, each with larger contiguous plots of 49 to 169 trees, for long-term crop experiments where yield and wood quality are of primary interest.
4. Arrangements of trees within plots in these two types of tests should differ. Plots containing few trees should be elongated parallel to suspected environmental gradients. Plots with larger numbers of trees should be square in shape to be most efficient.
5. Experimental blocks, to be as uniform as possible, should be small in size. Block size should decrease as the environmental gradient increases. Blocks much larger than 1 acre or $\frac{1}{2}$ hectare will probably include considerable environmental variation.
6. Evaluations for each character should include (a) analyses of variance and multiple range tests (Duncan, 1955; Kramer, 1956) to establish differences between provenances at each planting and interactions between provenances and plantation environments, (b) correlations between expression of a character in seed source tests and in native stands, (c) regressions of variation in the character on variation of climate and location at seed sources, and (d) trends in expression of the character over time, that is, juvenile-mature correlations (Schmidt and Stern, 1955). Techniques should be the same for measurements and observations in all plantings. Automatic data processing on computers should be used to simplify compilations and analyses.

Each seed source study must be conducted in at least two stages. In the first exploratory tests few to many provenances are tried on few to many sites. These first tests should point the way to the second stage. Refined tests are established using seed from good trees

⁷ Publications on results of seed source studies are too numerous to be summarized here. Anyone interested in the subject should refer to publications appearing during the last decade by Fielding (1953), Langlet (1953), Wakeley (1953), Lofting (1954), Rees and Brown (1954), Susmel (1954), Veen (1954), Johnsson (1955), Pauley *et al.* (1955), Lindquist (1956), Stoeckeler and Rudolf (1956), Kriebel (1957), Wright and Baldwin (1957), Boden (1958), Echols (1958), Wright (1960), Batzer (1961), Callaham and Liddicoet (1961), Bouvarel (1962), and Wright and Bull (1963).

⁸ Publications pertaining to the design of seed source studies are by Johnsson (1952), Strand (1952, 1955), Wright and Freeland (1960), Evans *et al.* (1961), and Shiue and Pauley (1961).

from the most promising sources planted at locations representing broad planting zones. Actually each of these stages may require several substages to accomplish the desired objects.

Seed source studies are costly because they require these steps. Considerable time must pass before performance can be evaluated. Fifty to 80 years might be required to find the best sources of fast-growing species, but undoubtedly some evidence of the better sources should begin to emerge in 10 to 20 years. One to four people might be employed to conduct tests of several species during this time. Sizable capital investments would be required for seed collection, for nursery growing of seedlings, and for plantation culture including land acquisition and fencing, tending and measuring of the trees, and for analysis and reporting of results.

Because provenance research is long term, some provision must be made to insure the protection of stands being tested. Particular attention should be given to the preservation and conservation of valuable sources of seed. Such stands should also be protected from chance hybridization with adjacent inferior stands.

Seed source studies — besides giving information on adaptability, growth and yield — can have additional value as a source of seed. Through the years that trees grow in seed source tests, many fail completely and others fall behind in growth. Only those adapted to the local conditions reach maturity. After judicious thinning to remove inferior individuals, the remaining trees may be propagated or progeny tested. Bouvarel (1958) has emphasized the desirability of using established successful stands of introduced species as seed sources.

Foresters establishing seed source studies might even consider establishing seed orchards concurrently with excess seedlings on areas of 2 to 4 hectares (5 to 10 acres). Single-tree plots might be established systematically in several blocks at a spacing of 2 by 2 meters (6 feet 6 inches $\times$ 6 feet 6 inches) and ultimately thinned to 10 by 10 meters (33 $\times$ 33 feet). The best of 25 progenies would be established at an open spacing favoring seed production.

Problems in semi-arid lands

Seed source studies are faced with unique problems in semi-arid lands where seasonal or annual moisture supply is deficient. The variation in precipitation about average values is of great importance. A 10 percent annual fluctuation in rainfall where moisture limits tree distribution is far more critical than a similar fluctuation in regions of abundant precipitation. Thus, slight climatic changes can be critical in semi-arid lands, for here trees grow near the threshold of survival.

This critical nature of climate demands close attention to experimental details. Experiments must be set up on representative sites where the environmental factors are known and can be measured during the course of experimentation. Observations must be repeated frequently during the first growing seasons to detect the timing and nature of limiting factors. Experiments in these lands are likely to require more replanting of failures to provide enough trees for meaningful measurement data.

The interaction of temperature and moisture which induces aridity makes the location of plantation areas critical. Extra consideration must be given to aspect, soils, water courses, and water-bearing strata. Minor differences in topography reflect major differences in climate and in soils. This results in greater difficulty in finding suitable sites for testing.

Semi-arid lands require more intensive experiments to study survival and more replication of field tests in time to encompass climatic changes. Annual climatic fluctuations are far more critical for trees growing near the survival threshold. Annual replications bring out the factors critical for establishment and survival.

Experiments in controlled environments may be more important than replications in subsequent years or location of replications. Climatic conditions in semi-arid lands cannot be very well predicted or modified. By controlling the environment, the investigator can test the reactions of plants to combinations of critical environmental factors.

References

- BATZER, H. O. *Jack pine from Lake States seed sources differ in susceptibility to attack by the white pine weevil*. U.S. For. Serv. Lake States For. Expt. Sta., Tech. Note 595. 2 p.
- BODEN, R. W. Differential frost resistance within one *Eucalyptus* species. *Aust. J. Sci.*, 21: 84-86.
- BOUVAREL, P. Variabilité de l'épicéa (*Picea excelsa* Link) dans le Jura français: répartition et caractères des divers types. *Rev. for. franc.*, 6 (2): 85-98.
- BOUVAREL, P. Les repeuplements artificiels - conséquences d'ordre génétique. *Jour. Forêts Sci.*, 8/9: 524-535.
- BOUVAREL, P. L'influence de l'origine des graines d'épicéa sur la croissance en pépinière; la précocité et la fréquence des pousses d'août. *Ann. de l'Ecole nationale des eaux et forêts*, 19 (3): 415-439.
- CALLAHAM, R. Z. Experimental taxonomy: more than seed source studies. In *Recent advances in botany*. Univ. of Toronto Press, p. 1695-1699.
- CALLAHAM, R. Z. Geographic variability in growth of forest trees. In T. T. Kozlowski, ed., *Tree growth*. New York, Ronald Press, p. 311-325.
- CALLAHAM, R. Z. & HSEL, A. A. *Pinus ponderosa*: height growth of wind-pollinated progenies. *Silvae Genet.*, 10: 33-42.

- CALLAHAM, R. Z. & LIDDICOET, A. R. Altitudinal variation at 20 years in ponderosa and Jeffrey pines. *J. For.*, 59: 814-820.
- CANADA. FORESTRY BRANCH. Tree breeding. In *Annual report on forest research, year ending March 31, 1960*. Dept. of Northern Affairs & Natural Resources, Forest Research Div., p. 19-20.
- CARLISLE, A. Variation in the native Scots pine of Scotland. In *Report on Forest Research for the year ending March 1954*. For. Comm., Gt. Britain, p. 55-56.
- CLAUSEN, J., KECK, D. D. & HEISEY, W. M. *Experimental studies on the nature of species. I. Effect of varied environment on western North American plants*. Carnegie Inst. of Washington, Pub. 520, 452 p.
- CLAUSEN, J., KECK, D. D. & HEISEY, W. M. *Experimental studies on the nature of species. III. Environmental responses of climatic races of Achillea*. Carnegie Inst. of Washington, Pub. 581, 129 p.
- CRITCHFIELD, W. B. *Geographic variation in Pinus contorta*. Harvard Univ., Maria Moors Cabot Foundation Pub. 3, 118 p.
- CYPRUS. FORESTRY DEPARTMENT. *Provenance trials of Pinus halepensis var. brutia*. 1953 report. 24 p.
- DOWNES, R. J. & PIRINGER, A. A., JR. Effects of photoperiod and kind of supplemental light on vegetative growth of pines. *For Sci.*, 4: 185-195.
- DUFFIELD, J. W. The importance of species hybridization and polyploidy in forest tree improvement. *J. For.*, 52: 645-646.
- DUNCAN, D. P. Multiple range and multiple F tests. *Biometrics*, 11: 1-42.
- ECHOLS, R. M. *Variation in tracheid length and wood density in geographic races of Scots pine*. Yale Univ., School of Forestry Bull. 64, 52 p.
- EDWARDS, M. V. The design, layout and control of provenance experiments. *Z. Forstgenet.*, 5: 169-180.
- EVANS, T. C., BARBER, J. C. & SQUILLACE, A. E. Some statistical aspects of progeny testing. *6th South. Conf. For. Tree Impr. Proc.*, 1961, p. 73-79.
- FIELDING, J. M. *Variations in Monterey pine*. Aust. For. Timber Bur. Bull. 31, 43 p.
- FIELDING, J. M. The role of exotic species in forest tree improvement. *Proc. 5th World For. Cong., Seattle, U.S.A.*, 2: 742-746.
- FUKAREK, P. Die Standortstrassen der Schwarzföhre (*Pinus nigra* Arn. sen Lat.). *Zbl. ges. Forstw.*, 75: 203-207.
- HABECK, J. R. White cedar ecotypes in Wisconsin. *Ecol.*, 39: 457-463.
- HESLOP-HARRISON, J. *New concepts in flowering-plant taxonomy*. Toronto, Heinemann, 135 p.
- HOPP, H. Multiple measures for distinguishing closely related plant forms. *Chron. Bot.*, 7: 402-403.
- HUXLEY, J. Clines: an auxiliary taxonomic principle. *Nature, Lond.*, 142: 219-220.
- IRGENS-MOLLER, H. Genotypic variation in photoperiodic response of Douglas-fir seedlings. *For. Sci.*, 8: 360-362.
- JACKSON, D. S. *Parameters of site for certain growth components of slash pine (Pinus elliottii Engelm.)*. Duke Univ., School of Forestry Bull. 16, 118 p.
- JOHNSSON, H. Einige Fragestellungen der forstlichen Nachkommenschaftsprüfung. *Z. Forstgenet.*, 2: 2-8.
- JOHNSSON, H. Utvecklingen i 15-åriga försöksodlingar av tall i relation till proveniens och odlingsort [Development of 15-year-old cultures of pine in relation to provenance and variety]. *Svenska Skogsv. Fören. Tidskr.*, 53: 56-88.
- KALELA, A. *Zur Synthese der experimentellen Untersuchungen über Klimarassen der Holzarten*. Metsät. Tutkimuslaitoksen Julkaisu. 26, 445 p.
- KRAMER, C. Y. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics*, 12: 307-310.
- KRIEBEL, H. B. *Patterns of genetic variation in sugar maple*. Ohio Agr. Expt. Sta. Res. Bull. 791, 56 p.
- LANGLET, O. Proviensförsök med olika trädslag [Provenance trials with various species]. *Svenska Skogsv. Fören. Tidskr.*, 1-2: 55-278.
- LANGLET, O. Nagra data från det internationella granproviensförsök av 1938 i Sverige [Some data on the 1938 international spruce provenance trial in Sweden]. *Skogen*, 40: 238-239, 244-245.
- LANGLET, O. A cline or not a cline: a question of Scots pine. *Silvae Genet.*, 8: 13-22.
- LANGLET, O. Central European spruce provenances in Swedish forestry. *K. Skogs- o. Lantbr. Akad. Tidskr. Stockh.*, 99: 259-329.
- LANGLET, O. Ecological variability and taxonomy of forest trees. In Kozłowski, T. T., ed. *Tree growth*, New York, Ronald Press, p. 357-369.
- LANGLET, O. Patterns and terms of intra-specific ecological variability. *Nature, Lond.*, 200 (4904): 347-348.
- LAWRENCE, G. H. *Taxonomy of vascular plants*. New York, 1951 Macmillan, 823 p.
- LEIBUNDGUT, H. Untersuchungen über das photoperiodische Verhalten von Fichtenkeimlingen. *Schweiz. Z. Forstw.*, 109: 520-523.
- LINDQUIST, B. Provenances and type variation in natural stands of Japanese larch. *Acta Horti Gotoburgensis*, 20: 1-34.
- LØFTING, E. C. Denmark's silver fir problem. Part I. Choice of provenances. *Det Forst. Forsøgsvæsen i Danmark*, 21: 377-381.
- MCMILLAN, C. Variation in seedlings of *Cupressus abramsiana* Wolf. *Madroño*, 12: 28-30.
- MCMILLAN, C. The edaphic restriction of *Cupressus* and *Pinus* in the coast ranges of central California. *Ecol. Monogr.*, 26: 177-212.
- MERGEN, F. & FURNIVAL, G. M. Discriminant analysis of *Pinus thunbergii* × *Pinus densiflora* hybrids. *Soc. Amer. For. Proc.*, 1960: 36-40.
- NIENSTADT, H. & OLSON, J. S. Effects of photoperiod and source on seedling growth of eastern hemlock. *For Sci.*, 7: 81-96.
- NYLINDER, P. & HAGGLUND, E. Standorts- och trädenskapers inverkan på utbyte och kvalitet vid framställning av sulfitmassa av gran [Effect of site and tree properties in yield and quality of Norway spruce sulphite pulp]. *Medd. Statens Skogsforskn. Stockh.*, 44 (11): 1-841.

- PAULEY, S. S. Variation in time of break of dormancy
1954 along altitudinal ecotypes of *Populus trichocarpa*.
Genet., 39: 986-987.
- PAULEY, S. S. & PERRY, T. C. Ecotypic variation of the
1954 photoperiodic response in *Populus*. *J. Arnold*
Arbor., 35: 167-188.
- PAULEY, S. S., SPURR, S. H. & WHITMORE, F. W. Seed
1955 source trials of eastern white pine. *For. Sci.*,
1: 244-256.
- PERRY, T. O. Racial variation in the day and night tem-
1962 perature requirements of red maple and lob-
lolly pine. *For. Sci.*, 8: 336-344.
- PHILIPPIS, A. DE. Varietà del *Pinus nigra* e reazione del
1937 suolo. *L'Alpe*, 24: 58-64.
- PRIEHÄUSSER, G. Über den Formenkreis der Fichte in
1956 ursprünglichen Beständen des Bayrischen Wal-
den nach den Zapfen- und Zapfenschuppen-
formen. *Z. Forstgenet.*, 5: 14-22.
- REES, L. W. & BROWN, R. M. Wood density and seed
1954 source in young plantation red pine. *J. For.*, 52:
662-665.
- ROHMEDEK, E. Problems and proposals for international
1952 forest tree seed certification. *Proc. 5th World*
For. Congr., Seattle, U.S.A., 2: 685-690.
- SCHMIDT, W. Die Sicherung von Frühdiagnosen bei lang-
1957 lebigen Gewächsen. *Züchter*, 4, suppl.: 39-69.
- SCHMIDT, W. & STERN, K. Methodik und Ergebnis eines
1955 Wachstumsvergleichs an vier zwanzigjährigen
Keifernversuchsflächen. *Z. Forstgenet.*, 4: 38-58.
- SCHREINER, E. J. Improvement of forest trees. U.S.
1937 *Dept. Agr. Yearbook*, 1937, p. 1242-1279.
- SCHÜTT, P. Zuchtung mit Kiefern. I. Individualunter-
1958 schiede und Provenienzversuche. *Mitt. d. Bundes-*
forschungsanstalt f. Forst- u. Holzwirtschaft, 40:
1-65.
- SHUE, C. J. & PAULEY, S. S. Some considerations on
1961 the statistical design for provenance and prog-
ny tests in tree improvement programs. *For.*
Sci., 7: 116-122.
- SNYDER, E. B. *Glossary for forest tree improvement workers*.
1959 New Orleans, La., U. S. For. Serv. South. Forest
Expt. Sta. 22 p.
- SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE. *Stand-*
1951 *ardized working plan for local tests of seed source*.
New Orleans, La., U.S. For. Serv. South. Forest
Expt. Sta. 11 p.
- SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE. *Work-*
1952a *ing plan for co-operative study of geographic*
sources of southern pine seed. New Orleans, La.,
U.S. For. Serv. South. Forest Expt. Sta. 35 p.
- SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE. *Test-*
1952b *ing tree progeny: a guide prepared by the Sub-*
committee on Progeny Testing. E. G. Wiese-
hügel, Chairman. Tenn. Valley Authority Tech.
Note 14. 21 p.
- SQUILLACE, A. E. & BINGHAM, R. T. Localized ecotypic
1958 variation in western white pine. *For. Sci.*, 4:
20-34.
- SQUILLACE, A. E. & SILEN, R. R. *Racial variation in*
1962 *ponderosa pine*. *For. Sci. Monogr.* 2. 27 p.
- STEBBINS, G. L., JR. *Variation and evolution in plants*.
1950 New York, Columbia Univ. Press. 643 p.
- STOECKELER, J. H. & RUDOLF, P. O. Winter coloration
1956 and growth of jack pine in the nursery as affect-
ed by seed source. *Z. Forstgenet.*, 5, 161-165.
- STRAND, L. Progeny tests with forest trees. *Hereditas*,
1952 38: 152-162.
- STRAND, L. Plot sizes in field trials. *Z. Forstgenet.*, 4:
1955 157-162.
- SUSMEL, L. Indagini sulla differenziazione del ritmo del-
1954 l'attività vegetativa in provenienze di *Abies alba*
Mill. *Ital. for. mont.*, 9: 289-302.
- SYLVÉN, N. Den nordiska tallen [The pines of northern
1916 Sweden]. *Medd. Statens skogsforsk. Inst. Stockh.*,
(13/14): 9-110.
- THORBJORNSEN, E. Variation patterns in natural stands
1961 of loblolly pine. *6th South. Conf. For. Tree*
Imp. Proc., p. 25-44.
- TURESSON, G. The genotypical response of the plant
1922 species to the habitat. *Hereditas*, 3: 211-350.
- TURESSON, G. The plant species in relation to habitat
1925 and climate. *Hereditas*, 6: 147-236.
- TURESSON, G. The selective effect of climate upon the
1930 plant species. *Hereditas*, 14: 99-152.
- VAARTAJA, O. Evidence of photoperiodic ecotypes in
1959 trees. *Ecol. Monogr.*, 29: 91-111.
- VEEN, B. General remarks on provenance research in
1954 forestry. *Euphytica*, 3: 89-96.
- VINOGRADOV, N. P. Mountain stands of *Pinus sylvestris*
1949 and the problem of afforesting chalk and lime-
stone exposures. *Priroda, Moskva*, 38 (9): 69-71.
(In Russian)
- WAKELEY, P. C. Progress in study of pine races. *Sth.*
1953 *Lumberman*, 187 (2345): 137-140.
- WASSINK, E. C. & WIERSMA, J. H. Daylength response of
1955 some forest trees. *Acta bot. neerland.*, 4: 657-670.
- WEIDENMANN, E. Die Versuche über den Einfluss der
1930 Herkunft Keifernsammes aus dem Preussischen
Forstlichen Versuchsanstalt. *Z. Forst. u. Jagd.*,
64: 498-522, 809-836. (English transl. by J. W.
Wright, Purdue Univ. 1944).
- WEIDMAN, R. H. Evidences of racial influence in a 25-
1939 year test of ponderosa pine. *J. Agric. Res.*, 59:
855-888.
- WETTSTEIN, W. Rassen- und Auchtungsforschung bei
1958 *Pinus sylvestris*. *Schweiz. Z. Forstw.*, 109: 495-505.
- WHITEHEAD, F. H. An example of taxonomic discrimina-
1954 tion by biometric methods. *New Phytol.*, 53:
496-510.
- WRIGHT, J. W. Genotypic variation in white ash. *J. For.*
1944a 42: 489-495.
- WRIGHT, J. W. Ecotypic differentiation in red ash. *J.*
1944b *For.*, 42: 591-597.
- WRIGHT, J. W. Geographic variation in forest trees. *2nd*
1960 *Cent. States For. Tree Imp. Conf. Proc.*, p. 15-20.
- WRIGHT, J. W. & BALDWIN, H. I. The 1938 International
1957 Union Scotch pine test in New Hampshire.
Silvae Genet., 6: 2-14.
- WRIGHT, J. W. & FREELAND, F. D. *Plot size and experi-*
1960 *mental efficiency in forest genetic research*. Mich.
State Univ. Tech. Bull. 280. 28 p.
- WRIGHT, J. W. & BULL, W. I. Geographic variation in
1963 Scotch pine: results of a 3-year Michigan study.
Silvae Genet., 12: 1-25.