Reducing the Effects of Nursery Influences Upon Provenance Tests

By Philip C. Wakeley

Southern Forest Experiment Station, Forest Service,
U. S. Department of Agriculture

THE RESEARCH worker who establishes a seed-source plantation is likely to find himself under almost immediate pressure to evaluate and publish the first observable results. These usually consist of differences in survival and height. One purpose of this paper is to show how variations in nursery environment can obscure the effects of various genotypes upon initial survival and early height growth. Further purposes are to list and document certain experimental procedures for minimizing such disturbing influences, and to explore one means of re-evaluating early survival when these procedures have not been followed.

Inconsistent Results With Seed From a North Carolina Source

At the First Southern Conference on Forest Tree Improvement, I commented on the good survival and growth of stock from the 1935 loblolly pine seed crop in Onslow County, North Carolina (Wakeley, 1951). In four plantations in South Africa (Sherry, 1947) and one each in Georgia and Texas, this stock had shown superior height growth for its latitude of origin (Figure 1).

Some Conference participants who had established a second, and later, loblolly pine provenance test challenged this evaluation of the Onslow County source. Their study included loblolly stocks of nine different geographic origins, compared in eight widely separated plantations in four southern states. In the words of a note published the year following the Conference, the Onslow County stock "failed utterly in all localities" (Cummings, 1952).

In the Southwide Pine Seed Source Study, Onslow County loblolly was tested a third time, with seed from

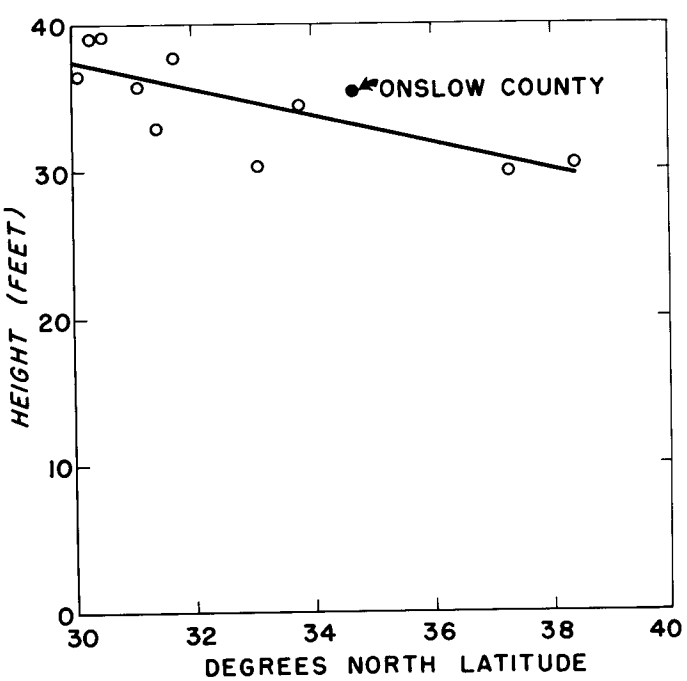

Figure 1—Regression of mean heights of 11 provenances of loblolly pine (Sherry, 1947) over latitudes of U. S. seed sources, after 9 years in plantation at Border, Natal, South Africa. Here, as in three other plantations in South Africa and two in the U. S., the Onslow County source lies above the curve.

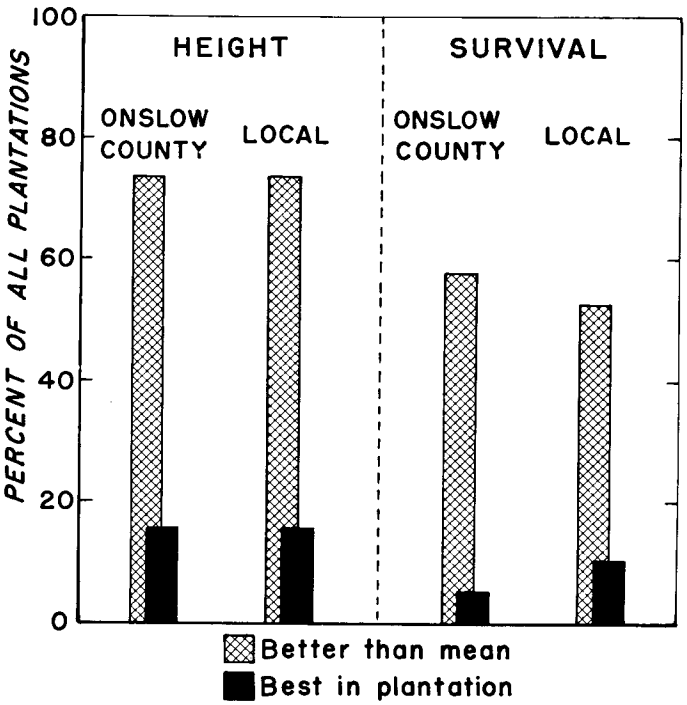

Figure 2—Relative heights and survivals of Onslow County and of local loblolly pine stocks, at 5 years, in 19 plantations of the Southwide Pine Seed Source Study.

the 1951 crop. Stock was planted in 19 localities from Maryland to southern Georgia and west to Arkansas and Texas (Wakeley, 1959). In each plantation, Onslow stock was compared with seedlings representing seven or eight other geographic sources.

In a majority of the 19 plantations, both Onslow County stock and the various "local" stocks from the geographic sources nearest the planting sites showed better than average five-year survival and growth. In a few plantations either Onslow or local stock survived or grew better than any other. Figure 2 shows the percentages of all plantations in which the Onslow and the array of local stocks attained these two levels of superiority. (Onslow was itself the local stock in two plantations.) Onslow compares favorably with local stock in both growth and survival in these 19 plantations distributed over the range of the species. Furthermore, in the Dooly County, Georgia, plantation (Figure 3) and in several others, Onslow stock exhibited the same superiority of height for its latitude of origin that it had shown in the South African, Georgia, and Texas plantations exemplified in Figure 1.

In the first and third studies, all the different stocks for any one plantation had, as a rule, been produced in a limited area within some one nursery. In the second study, no two stocks were grown under identical nursery conditions. Four were grown in different parts of one nursery. The other five, including the Onslow stock, were grown in different parts of a second nursery that had variable soil and produced stocks of widely variable average dimensions. As later appeared, the stocks from this second nursery also varied greatly in survival. Taken together, these facts suggest strongly that the Onslow stock in the second study failed because of some adverse nursery influence, not because of its genotype.

How Variations in Nursery-induced Physiological Quality Affect Results

It has been amply demonstrated that nursery influences other than the obvious ones of root breakage, root exposure, poor packing, and the like can affect initial survival of planted conifers. Seedlings of the same genotype, grown at uniform density from simultaneous sowings and carefully matched in size, often differ significantly in early survival, and sometimes in early growth. The differences appear to be attributable to differences in physiological quality resulting from variations in nursery soil texture, nutrients, or moisture relations (Lavender and Wright, 1960; Stone, 1955; Stone and Benseler, 1962; Stone, Jenkinson, and Krugman, 1962; Stone and Schubert, 1959a and 1959b; Wakeley, 1949; Wakeley, 1954, pp. 102-110 and 122-123).

The following example illustrates the effects of nursery influences upon survival.

Identical lots of loblolly, slash, and longleaf pine seed were sown within three days of each other in two nurseries. The seedling stands of each species were thinned to appropriate uniform densities, which were maintained throughout the growing season. Seedlings were outplanted in replicated, balanced, randomized blocks. Figure 4 shows the first-year plantation survivals. The striking species x nursery interaction demonstrates how results can be affected by differences in physiological quality induced by nursery influences other than seedbed densities. If, in a provenance test, longleaf from one geographic source had been grown in nursery X and that from another in nursery Y, the effect of nursery environments would almost surely have obscured any effect of genotype upon initial survival.

A second example illustrates the effects of nursery influences on early height growth.

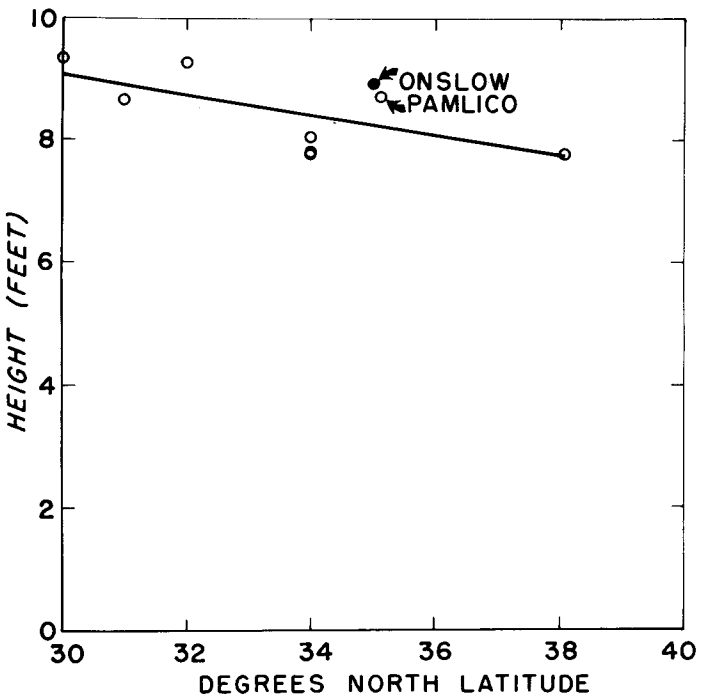

Figure 3—Regression of mean heights of 9 provenances of loblolly pine over latitudes of seed sources, after 5 years, in a Dooly County, Georgia, plantation. The superior position of the Onslow County source is similar to that in Figure 1.

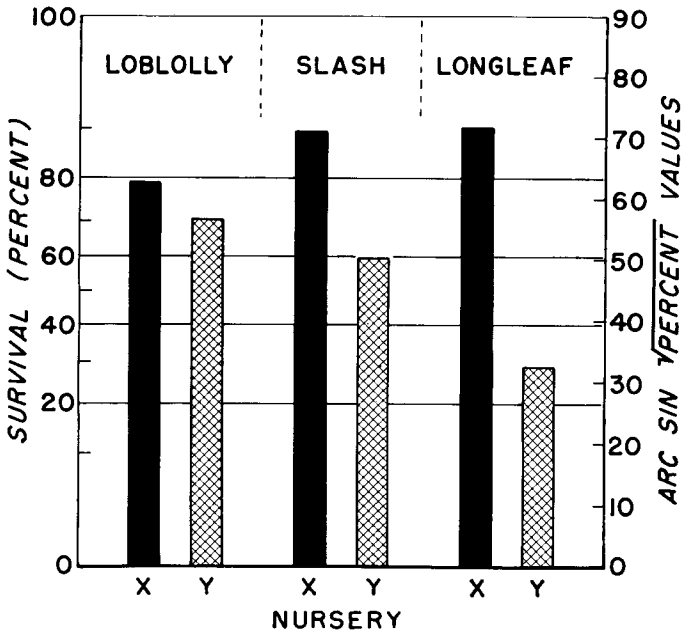

Figure 4—First-year plantation survivals of pine seedlings grown from the same seed lots in two different nurseries.

Two locations in nursery A were sown with loblolly seed of the same lot. Four other locations, two each in nurseries W and M, were all reportedly sown with seed of a second lot, distinct from but of the same general provenance as the first.

From beds of comparable density in each of the six nursery locations, 200 large seedlings precisely matched in size, and 200 similarly matched[1] small seedlings, were lifted and divided equally among replicated, balanced, randomized blocks on two planting sites.

Four years after planting, the mean height of the stock from the best nursery location exceeded that from the poorest by 1.24 feet on site 1 and 1.26 on site 2 (i.e., by 20.6 and 23.6 percent) (Figure 5). Differences were significant at the 1-percent level. More important, despite origin of the seedlings from the same seed in each case, height differences significant at the 5-percent level had developed even between location-1 and location-2 stocks of nursery A on site 1, and between location-1 and location-2 stocks of nursery W on site 2.

Designing Studies to Minimize Disturbing Nursery Effects

Care in lifting, packing, storing, and transporting the stock can minimize or eliminate the disturbing effects of such nursery accidents as root-breakage during lifting, desiccation of seedlings, and the like. The more subtle effects of differences in physiological quality, on the other hand, can rarely be eliminated by mere care in nursery practice, because their underlying causes are generally obscure or unknown. Minimizing or eliminating nursery effects of this second type depends, therefore, upon sound experimental design of the nursery phase of the provenance test.

"Minimum Standards for Progeny-testing Southern Forest Trees for Seed-certification Purposes" (Wakeley, *et al.*, 1960)[2] gives in full the specifications for nursery design paraphrased here. These specifications are as applicable to provenance tests as to any other progeny tests. As numbered in the reference just cited, they are:

13. Seedlings to be planted for provenance tests must be grown together on soil as uniform as can possibly be obtained, or, if they are grown on different soils, must be so distributed that like proportions of all progenies are produced on each distinct class of soil. The standard specially warns against growing one progeny in one nursery and a second in another nursery.

14. The assignment of seed sources to individual plots within the nursery area or areas must be at random.

15. Seedlings to be used in provenance tests must be from seed lots sown at approximately the same time—within a very few days of each other at most. They must be grown as nearly as possible under identical conditions of soil treatment (including prior cover crops, soil prepara-

[1] Except that the small seedlings from location 1 of nursery M were all 6.6 to 7.5 inches tall. All other small seedlings were 7.6 to 8.5 inches tall. All large seedlings were 9.6 to 10.5 inches tall. In the plantations, the 6.6- to 7.5-inch seedlings did not differ significantly in growth from the 7.6- to 8.5-inch seedlings from the other location in the same nursery.

[2] The standards, prepared by a special Subcommittee of the Committee on Southern Forest Tree Improvement, may be obtained either from the Southeastern Forest Experiment Station, Asheville, North Carolina, or the Southern Forest Experiment Station, New Orleans, Louisiana.

Table 1.--*Reduction of differences between mean ranks of similar loblolly pine stocks and of replications of identical stocks through use of fifth-year ÷ first-year survival*

Sources compared	Gross fifth-year survival		Fifth-year ÷ first-year survival	
	Mean rank 1/	Difference	Mean rank 1/	Difference
1. Two nearly adjacent Coastal Plain counties				
C-303, Onslow County, N. C., in Series 1	4.22	2.22	4.67	1.11
C-305, Pamlico County, N. C., in Series 1	6.44		5.78	
2. Two Piedmont counties with similar climates				
C-311, Clarke County, Ga., in Series 2	4.00	1.00	6.11	1.22
C-331, Spalding County, Ga., in Series 2	5.00		4.89	
3. Two nearest interior Alabama counties				
C-317, Clay County, Ala., in Series 1	6.67	1.89	7.11	1.00
C-319, Jefferson County, Ala., in Series 2	4.78		6.11	
4. Two sources with driest climates				
C-325, Angelina County, Tex., in Series 1	3.56	1.00	3.56	.34
C-327, Clark County, Ark., in Series 1	2.56		3.22	
5. Two sources most alike in low latitude and high temperature, rainfall, and humidity				
C-309, Wilcox and Crisp, Ga., in Series 1	6.44	.88	6.00	1.33
C-323, Livingston Parish, La., in Series 1	5.56		4.67	
6. Two northernmost sources in Series 1				
C-301, Somerset County, Md., in Series 1	5.11	.89	4.56	.11
C-303, Onslow County, N. C., in Series 1	4.22		4.67	
7. Two most northeasterly sources in Series 2				
C-303, Onslow County, N. C., in Series 2	5.11	1.44	5.44	1.00
C-307, Newberry County, S. C., in Series 2	3.67		4.44	
Identical sources replicated in 2 series of plantations:--				
8. C-303, Onslow County, N. C. — In Series 1	4.22	.89	4.67	.77
8. C-303, Onslow County, N. C. — In Series 2	5.11		5.44	
9. C-323, Livingston Parish, La. — In Series 1	5.56	1.11	4.67	.44
9. C-323, Livingston Parish, La. — In Series 2	6.67		5.11	
10. C-327, Clark County, Ark. — In Series 1	2.56	1.44	3.22	1.22
10. C-327, Clark County, Ark. — In Series 2	4.00		2.00	
Total, all 10 comparisons	--	12.76	--	8.54
Mean	--	1.276	--	0.854
Difference between analytical techniques		2/0.422		

1/ Each value based on 9 plantations
2/ Significant at 5 percent level

tion, fumigation, fertilization and soil amendment), watering, cultivation, late-season fertilization, and seedbed density. The requirement concerning seedbed density will often necessitate thinning to uniform density while the seedlings are in the primary-needle stage.

16. For any one test plantation, the lifting, packing, storage, and transportation of all the progenies must be the same, and must be as nearly simultaneous as possible for all the progenies.

17. If the seedlings are graded rather than selected at random from the whole nursery population of each progeny, the utmost care must be taken that the grading itself does not bias results for or against any given progeny.

In connection with Standard 14—random assignment of progenies to nursery plots—the "Minimum Standards" make the following additional recommendations, which I should like to emphasize by quoting verbatim:

> It is recommended that each progeny be sown in two or more random replications in the nursery to average out the effects of any variations in the nursery environment. If the test plantation design is to consist of balanced randomized blocks and is to evaluate characters capable of being influenced by nursery conditions, it is further recommended that nursery sowing be in an equal number of balanced randomized blocks and that nursery blocks be confounded with plantation blocks. This is done by assigning each nursery block at random to a different plantation block, and planting each plantation block exclusively with stock from the nursery block thus paired with it.

This device of confounding nursery blocks with plantation blocks has been tried extensively and found highly effective in nursery studies requiring plantation follow-up for observation of survival and growth. It preserves, and removes from the total sum of squares, the sum of squares for differences developing among blocks in the nursery, instead of obscuring it as when seedlings from nursery replications are composited. It appears to be the best procedure yet devised for minimizing the disturbing effects of variable physiological qualities upon provenance-test plantations.

Suggestion for Factoring Out Disturbing Nursery Effects on Survival

In provenance tests generally, it can hardly be questioned that accidents during lifting and planting do sometimes obscure the effects of genotypes upon gross survival—that is, upon number of trees alive over number planted. Can the difficulty be overcome by some alternative mode of expressing early survival? And if it can be overcome in the case of nursery accidents, may it perhaps also be overcome when nursery-induced differences in the physiological quality of various stocks similarly obscure the effects of genotype? An analytical technique for separating the effects of physiological quality from those of genotype would be especially helpful when the canons of experimental design outlined in the preceding section had not been strictly observed.

Apparently little research has been done on such alternative methods of expressing early survival. A method has been formulated, however, on the assumptions that: 1) Such accidents as root breakage or root exposure affect survival almost entirely during the first year after planting; 2) even poor physiological quality generally takes its greatest toll within one year; and therefore, as a rule, 3) the influences

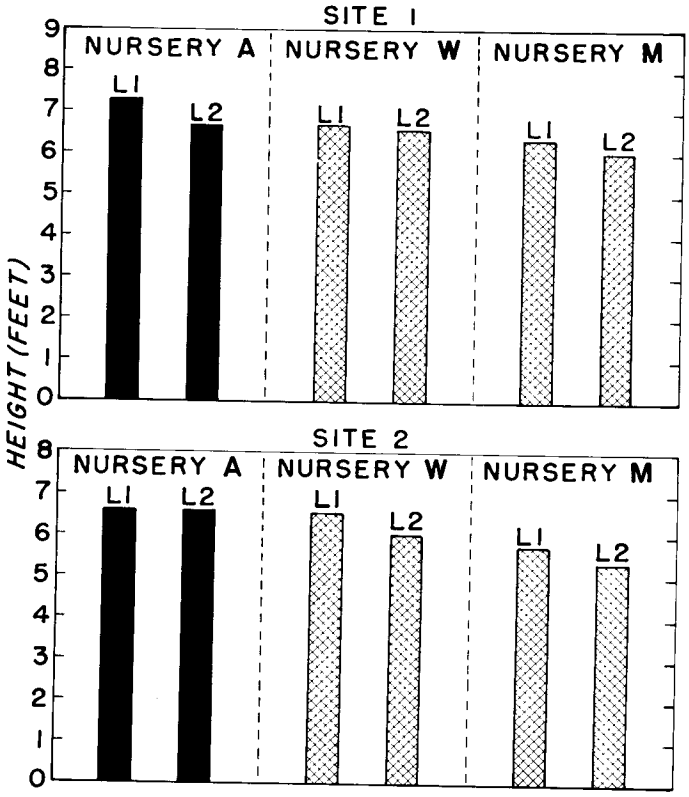

Figure 5—Mean heights of loblolly pines, four years after planting, on two sites, by nurseries and locations (L) within nurseries. Stocks known or reported to be from the same seed lot are indicated by identical symbols.

of stock genotype and plantation environment eventually offset the early effects of nursery accident and nursery environment. (In special cases, as E. B. Snyder shows elsewhere in these proceedings, the aftereffects of nursery influences may persist indefinitely.) To utilize the generally increasing ascendency of genotype and of plantation environment, it is proposed to evaluate different stocks in provenance tests in terms of fifth-year or other later survival over first-year survival.

This analytical technique still requires extensive testing, and may have to be modified to fit particular localities or species. Nevertheless, a preliminary application to data from 18 loblolly pine plantations in the Southwide Pine Seed Source Study (fairly well distributed over the commercial range of the species from Maryland to Texas) seems worthy of mention.

Each of the 18 plantations contains stocks representing eight or nine geographic sources.

The mean survivals of the stocks in each plantation were arranged in descending order. Then the mean rank of each stock was calculated from all plantations in which that stock appeared. This was done both in terms of gross survival at five years, and of fifth-year over first-year survival.

It was taken as a reasonable hypothesis that the survivals of stocks representing similar geographic origins should have similar ranks in a given set of plantations. For example, stocks from Onslow and Pamlico Counties, which are nearly adjacent in the North Carolina Coastal Plain, should have similar survival patterns, just as they exhibited

simalar height patterns in Figure 3. Again, there were three stocks that were common to two otherwise different sets of stocks under observation in two series of nine plantations each. It was assumed that one such stock having a high rank in one series of plantations should also have a high rank in the other series.[3] In all, 10 such comparisons were set up (Table 1).

In terms of gross survival at five years, the mean ranks of some of these paired similar sources differed considerably. Even the replicate plantings of stocks from the same seed lot had disconcertingly different ranks.

When the results were expressed in terms of fifth-year over first-year survival, similarity of mean rank was increased for eight of the 10 pairs (Table 1). In some instances the increase in similarity was very marked. Analysis of variance showed that for the 10 comparisons as a group, the improvement was significant at the 5-percent level.

SUMMARY

Variations in nursery environment can obscure the effects of genotype on initial survival and early growth in provenance tests. The nursery phases of the tests can and should be designed to minimize such disturbing influences.

Even when nursery design has not been of the best, evaluation of genotypic effects upon early survival may sometimes be improved by analyzing in terms of later-year over first-year survival rather than of gross survival.

[3] This assumption would not be valid for grossly dissimilar series, but seems justified for the two series considered here, as they represent fairly comparable geographical ranges of seed sources and plantation locations. Series-1 sources and locations extend from north latitude 30° 00′ to 38° 07′, and from west longitude 75° 28′ to 95° 30′. The corresponding figures for Series 2 are north latitudes 30° 00′ and 35° 07′, and west longitudes 77° 00′ and 93° 00′. Furthermore, four of the nine plantations in Series 1, in North Carolina, Mississippi, Louisiana, and Arkansas, are immediately adjacent to four of the nine in Series 2.

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Wood Properties of Clones of Slash Pine

By Bruce Zobel, Don Cole and Roy Stonecypher

Professor, Forest Genetics, N. C. State College, School of Forestry, Raleigh; Research Forester, Continental Can Company, Savannah, Georgia; Graduate Student, School of Forestry, N. C. State College, Raleigh, respectively.