Literature Cited

CONKLE, M. T., W. J. LIBBY and J. L. HAMRICK: Winter injury among white fir seedlings. USFS Research Note PSW 138, 7 p. (1967). — GATHY, P.: A propos de l'hybride nature1 Abies concolor (GORD.) ENGELM. X Abies grandis LINK. Silvae Genetica 6: 186–190 (1957). — HOPKINS, A. D.: Bioclimatics, a science of life and climate relations. USDA Misc. Pub. 280 (1938). — LAMB, W. M.: A conspectus of North American firs (exclusive of Mexico). Soc. Am. Foresters Proc. 9: 528–538 (1914). — LARSEN, C. S.: Genetics in silviculture (translated by M. L. ANDERSON). Oliver and Boyd, London, 147–149 (1956). — MÜLLER, K. M.: Abies grandis and its climatic races. Mitteilungen der Deutschen Dendrologischen Gesellschaft. 1939, 136 p. — SCHEPLITZ, B.: Über einen natürlichen Abies-Bastard. Morphologische und holztechnologische Untersuchung an Artbastarden von Abies concolor x Abies grandis. Zeit. Forstgenet. 5: 71–79 (1956). — WRIGHT, J. W., and BULL, W. I.: Geographic variation in European black pine — two year results. For. Sci. 8: 32—42 (1962). — ZAVARIN, E., and SNAJBERK, K.: Chemotaxonomy of the genus Abies. I. Survey of the terpenes present in the Abies balsams. Phytochemistry 4: 141–148 (1965).

Crossing Among Loblolly Pines Indigenous to Different Areas as a Means of Genetic Improvement

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Introduction

Of the several plant breeding procedures that can be used to improve outcrossing species, the one currently most employed in first generation selection of loblolly pine (*Pinus* taeda L.) is mass selection within locally adapted stock. This breeding method is applicable because there are large amounts of between-tree and within-population variability of a genetic nature.

Economically important differences also exist among geographic races within this species. WELLS and WAKELEY (1966) reported results for loblolly pine that indicated there may be benefits from using non-local seed sources in certain areas. Among their results were: (1) trees of western origin and from one far northeast area were consistently least infected with fusiforrn rust; (2) in practically all plantations, trees of western origin survived best; (3) in all but the coldest location, the trees from coastal areas grew fastest.

These and similar results indicate that hybridization among trees indigenous to different areas might be an additional breeding method for loblolly pine. First generation among-area crosses (wide crosses) conceivably could combine parental characteristics thus producing new genotypes having superior growth performance, drought or cold hardiness, or disease resistance. Likewise, new genetic combinations which would be useful in later breeding cycles could be expected to be created.

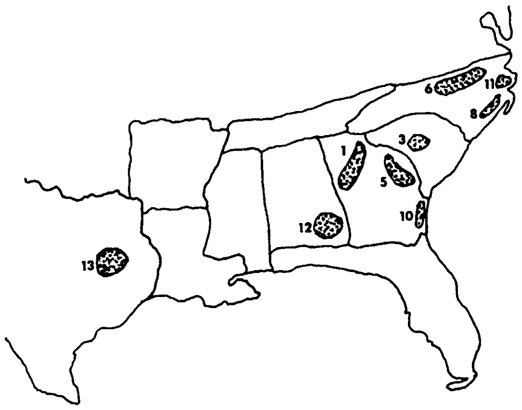
The results reported here are from a juvenile assessment of loblolly pine crosses among seed orchard selections indigenous to three different areas of the Southern United States: the Coastal Plain, the Piedmont, and west of the Mississippi River.

In a juvenile analysis, gerrnination characteristics of sound seed were evaluated to see if there were any indications of genetic incompatibilities resulting from wide crossing. A measure of first year height growth hybrid vigor was obtained by evaluating a control cross-pollinated family as a percent of the wind-pollinated families collected from the parental clones. Duration and distribution of growth were assessed to see if hybrids of North Carolina sources with more southern sources would have a period of physiological activity that would allow them to be grown at latitudes more northern than the origin of their most southern parent.

Materials and Methods

Parental Population

Control pollinations for the wide crosses were made according to mating design II of COMSTOCK and ROBINSON (1948). Each of several seed orchard clones used as female parents were pollinated by a set of clones used as male parents. The geographic origin of the ortets is shown in Figure 1. Four female parents of Piedmont origin were crossed with 5 males from the Piedmont, ten males from the Coastal Plain, and 4 males of Western origin. Four female parents of Coastal Plain origin were crossed with



	Source Number	Number of Clones
FEMALE PARENTS		
Coastal Plain Source	8	4
Piedmont Source	6	4
MALE PARENTS		
Coastal Plain Sources	11	4
	8	4
	10	2
Piedmont Sources	3	2
	5	4
	1	2
	12	3
Western Source	13	4

Figure 1. — Southern states showing origin of the trees used as female and male parents. The dark lines delineate the areas within which the ortets were selected.

Table 1. — Wide crosses by female and male parent combinations (See *Figure 1* for parental locations).

Male Parent No.	Female Parent			
	Piedmont			
	6—7	6—9	6—10	6—20
Piedmont				
5—19	×	×	×	×
5—41	×	×	×	×
12—7	×	×	×	×
12—12	×	×	×	×
12—13	×	×	×	×
Coastal				
11—502	×	×	×	×
11—504	×	×	×	×
11—510	×	×	×	×
11—532	×	×	×	×
10—6	×	×	×	×
10—37	×	×	×	×
8—30	×	×	×	×
8—33	×	×	×	×
8—68	×	×	—	×
8—76	×	×	×	×
Western				
13—1	×	×	×	×
13—2	×	×	×	×
13—6	×	×	×	×
13—8	×	×	×	×
	Coastal Plain			
	8—30	8—33	8—68	8—76
Piedmont				
1—10	×	×	×	×
1—14	×	×	×	×
3—8	×	×	×	×
3—20	×	×	×	×
5—5	×	×	×	×
5—12	—	×	×	×
Western				
13—1	×	×	×	×
13—2	×	×	×	×
13—6	×	×	×	×
13—8	×	×	×	×

6 males from the Piedmont, and 4 males of Western origin. *Table 1* shows the successful wide crosses.

Within area material to serve as a basis of comparison with the wide crosses was obtained by two means. Wind pollinated seed was collected from 32 seed and pollen parents. Also the clones used as female parents were used as males in a diallel crossing scheme. This yielded 24 control cross-pollinated families; 12 for the Coastal source females and 12 for the Piedmont source females.

Environmental Design

The experimental trees were grown as tubed seedlings in 12 replication randomized complete block designs under two different environmental regimes near Raleigh, North Carolina. The families which were common to both environments allowed evaluation of the performance of the same wide crosses under radically different growing conditions. The main study consisting of (1) all the wide crosses, (2) the within-area diallel matings (3) and the wind-pollinated families allowed evaluation of the seedlings under near ideal growing conditions. The main study was grown in a fertile, well-watered loamy soil. The stress study consisting of (1) a sample from the Coastal Plain and Piedmont wide crosses and (2) wind-pollinated lots of the wide cross parents allowed evaluation of the seedlings under stringent environmental conditions. The stress study was grown on a sandy soil low in nutrients which was watered only infrequently.

Table 2. — Form of analysis of variance on a plot mean basis for analysis of area differences for the wide-cross and within-area crosses.

Source of Variation	MS	F
replications	M_1	
areas	M_2	M_2/M_3
families in areas	M_3	M_3/M_4
error	M_4	

The pollen parents used in the stress study, those from Grimes County, Texas, and Tyrell County in Coastal North Carolina, were from very diverse natural environments. The Texas trees had been selected on droughty soils, whereas the North Carolina trees were growing on wet, deep peat soils.

Data Analysis

The analysis for detection of area differences *(Table 2)* was used in both the main and stress studies. Areas were designated by the geographic source of the pollen parent for the wide crosses. The within-area crosses created by the diallel matings were analyzed as separate areas.

Results

Seed Germination

Percentage germination of all experimental materials was quite high with an overall average greater than 95%. An analysis of variance of area differences indicated no statistically significant differences among areas for percentage germination. However, individual families within areas were highly significantly different in all analyses *(Table 3)*.

There were also no indications that wide crossing results in production of abnormal seedlings. Abnormal seedlings resulted from less than 1% of the 21,000 seeds planted and these few were equally distributed among the wide crosses, within-area crosses, and wind-pollinated families.

Table 3. — Analysis of variance results for percentage germination and total 1 year height as a percent of the open-pollinated progenies of the two parents.

Source of Variation	Degrees of Freedom	Level of Significance	
		Germination	Height as %
		MAIN STUDY	
Piedmont Females			
replications	11		
areas	3	>5%	>5%
families in areas	83	1%	1%
error	1090		
Coastal Females			
replications	11		
areas	2	>5%	1%
families in areas	45	1%	5%
error	517		
		STRESS STUDY	
Piedmont and Coastal Females			
replications	11		
areas	2	>5%	>5%
families in areas	45	5%	1%
error	517		

The results obtained for germinating characteristics indicate that no reduction in seed germination would be obtained from wide crossing within loblolly pine. Therefore, seed orchards could be established with parents from different seed orchard areas just as they are now established for local populations within any given area, i. e., if they flower at the same time.

Height

The data for the wide crosses and within-area crosses were analyzed as a percent of the mean of the wind-pollinated families obtained from the parental trees used in making the wide crosses. This enabled determination of the percentage of heterosis as a deviation from mid-parent values.

The major result in the main study was the lack of superiority of the wide crosses relative to the within-area crosses *(Tables 3 and 4)*. Just as large a positive heterotic response was shown by the within-area crosses, while the largest negative heterosis was obtained when western pollens were used. This indicates there was little positive heterosis on an area basis from making such crosses. Just as with germination data, the largest and most frequent statistically significant differences existed among crosses within areas rather than between areas *(Table 3)*. Individual wide cross families approached the performance of the best within-area crosses as can be seen by the range of values for the wide crosses *(Table 4)*.

In the stress study, the largest height superiority (7.6%) was for Piedmont female × Coastal male crosses, and the smallest (0.3%) for the Coastal female × Western male crosses. It was noteworthy that the mean of the Coastal and Piedmont female wide crosses resulting from pollination by the Western trees now had a slight 2.3 percent superiority over the mid-parent height values, whereas in the main study, they were 4.3 percent inferior.

Not only did the highest percentage superiority occur in the stress study, but the wide crosses resulting from the use of Western pollens changed from a negative heterosis in the main study to a positive heterosis in the stress study. These results indicate that the more dissimilar the environmental conditions from those in which the parents originated, the more superior the performance of the wide crosses judged relative to the mid-parent value. Similar results in maize were found by McWilliam and Griffing (1965) for they found additional heterosis in hybrids when the plants were grown at temperatures above their growth optimum. The temperature-dependent heterosis was manifest because the hybrids grew relatively better than the parents at high or low temperatures and recovered more rapidly after heat shock treatments. Crosses between races of mimulus were found by Hiesey (1965) to be suited to environments unsuitable for either parent.

Seasonal Distribution of Growth

Examination of the seasonal distribution of growth of the wide crosses indicated that seed source, genetic combination, daylength, soil moisture availability and possibly other factors interacted to produce distinctive growth patterns. Of relevance here is the fact that parental growth

Table 4. — Height of the 1-year-old wide-cross families as a percent of the open-pollinated progenies of the two parents.

Cross	Height as Percent of Mid-parent Mean		
	Mean	Standard Error	Range
		MAIN STUDY	
Piedmont Females			
× Piedmont males	4.6		—2.9 to +15.4
× Coastal males	1.7		—6.8 to + 6.7
× Western males	—3.8		—7.1 to + 0.1
Within-area crosses	4.3		—5.1 to +16.0
mean	+1.7		
Coastal Females			
× Piedmont males	1.3	±2.5	—1.7 to + 3.9
× Western males	—4.5	±2.5	—7.6 to + 2.3
Within-area crosses	3.1	±5.5	+1.3 to + 4.8
mean	0.0		
		STRESS STUDY	
Piedmont Females			
× Western males	4.3		—5.1 to + 8.7
× Coastal males, source II	7.6		+3.1 to +19.9
Coastal Females			
× Western males	0.3		—5.9 to + 5.6
mean	+4.1		

Table 5. — Comparison of periodic growth rates of within and between-source crosses.

Cross	Height Increments (Centimeters)				
	0—7 weeks	7—15 weeks	15—27 weeks	27—45 weeks	Total Height 45 weeks
Piedmont × Piedmont	12.5	10.0	7.4	6.5	36.4
Piedmont × Coastal	12.2	10.1	8.1	6.7	37.1
Coastal × Coastal	14.1	12.0	7.4	5.9	39.4

patterns were combined in the wide cross families indicating that potentially useful genetic combinations were created in the F_1 crosses.

The contrast of the growth patterns of the Coastal source seed parents and Piedmont source seed parents is an interesting example of this because both sources occur at essentially the same latitude. Yet, as can be seen in *Table 5*, the within-area Coastal crosses grew faster at the beginning of the growing season than the within-area Piedmont crosses; however, the Coastal source grew slower in the 27—45 week period. If the early growth pattern of the Coastal source could be combined with the later sustained growth of the Piedmont source, crosses between the two might be quite superior on certain types of sites. Something of this nature did happen; the crosses between the two areas lagged behind the Coastal seed parents by 1.9 cm in the initial 0—7 week growth periods, but were essentially equal to the Piedmont parents. In the last two growth periods, the between-area crosses exceeded the mean of the within-area crosses by .6 cm reaching a height of 37.1 cm which placed it midway between the 39.4 cm of the Coastal source and the 36.4 of the Piedmont source.

Duration of Growth

Observations made on the plants when grown at Raleigh, North Carolina, under normal day length and temperature for that latitude indicated that there were as much as eight to ten week differences among families in duration of growth. Just as with seasonal distribution of growth, several factors interacted to determine the length of the growing season. Of utmost importance, though, is the fact that no family sustained any damage from fall frosts. If the wide crosses prove hardy to spring frosts, freeze damage and snow breakage, the better performing wide crosses can be planted in latitudes more northern than the latitude of origin of the most southern parent.

Agreement with Results from Previous Studies

The results obtained in this juvenile assessment of crosses among seed orchard trees indigenous to different geographic locations give no indication that any heterotic response of the type found for yields in varietal crosses of crop plants (15—25% over the mid-parent value) is manifested in height growth response of F_1 wide crosses of loblolly pine populations. Reports of observed heterosis of within-species crosses in the pines involving a large number of parents are not yet available, if heterosis is defined as increased performance of the F_1 hybrid over wind- or control-pollinated material taken from both of the parental trees. Results reported in forestry frequently involve comparison with only one parent (Nilsson, 1963; Orr-Ewing, 1966) or comparison with the parental provenances (Johnsson, 1956). Ehrenberg (1966) used both parents as a basis of comparison but had only one cross. Since there is meager evidence of a sizable heterotic growth response in the pines resulting from between population crosses, the results for loblolly pine reported here can be considered to be typical.

Discussion

Although little heterosis in the classical sense was found there were indications that such crosses have potential use in forestry. Evidence of height growth superiority of the wide cross group was negligible when planted in a good soil. However, under poorer growing conditions, the wide cross groups changed from a negative to a positive growth response. This indicates some wide crosses would be suitable for planting on a more diversified range of site conditions than their parents.

The possible gains to be made in wide crossing by taking advantage of the frequently reported better growth potential of Coastal Plain loblolly stock and the drought tolerant characteristics of the Piedmont stock should not be overlooked. In this study, Coastal × Piedmont crosses from the same latitude were intermediate to their parents. If this performance is maintained under typical Piedmont growing conditions, the advantages of this cross as opposed to using Coastal sources would be considerable since there would not be as great a danger of poor survival during occasional adverse droughty years.

The greatest possible potential of the wide crosses with the Western sources is yet to be tested. Trees from the Western portion of the loblolly range have been found to be only lightly infected with fusiform rust. If a part of this resistance is carried in the wide crosses, gain in yield per acre over local stock could reach figures comparable to gains made in crop plants. Likewise, the higher survival that might result from crosses with Western sources needs to be tested under severe natural conditions.

The likelihood of using wide crosses to broaden and diversify the genetic base of the next generation or future generations of seed orchards is of major importance. The observations made on the F_1 population may prove to be an inadequate guide to performance in later generations since only those loci at which alleles are not fixed are presently contributing to within and between population variability. These loci contribute to the variation in subsequent random mating generations and may prove to be a source of many new utilizable genetic combinations.

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Summary

A series of wide crosses were made using as parents seed-orchard selections of loblolly pine indigenous to three different geographical areas of the southern United States: the Coastal Plain, the Piedmont, and west of the Mississippi River. First year height growth measurements revealed little area by area heterosis in the wide-cross families under good growing conditions when evaluated as a percent of the wind-pollinated families taken from the parental trees. A larger area by area heterosis for first year total height was found under conditions of restricted moisture and nutrients. Analysis of variance of area differences indicated that height differences attributable to areas were small and few were statistically significant. The bulk of the variability was attributable to individual wide-cross families within pollen source areas. Although little heterosis for height growth was observed in the wide crosses on an area basis, the performance of some individual wide-cross families indicates new desirable genetic combinations have been produced.

Literature Cited

Comstock, R. E., and H. F. Robinson: The components of genetic variance in populations. Biometrics 4: 254—266 (1948.) — Ehrenberg, C.: Parent progeny relationship in Scots pine (*Pinus silvestris* L.). Studia Forestalia Suecica Nr. 40: 1—54 (1966). HIESEY, W. M.: The genetic-physiologic structure of species complexes in relation to environment. Proceedings 11th International Congress Genetics 1963. — Pergamon, Oxford, 1965. — JOHNSSON, H.: Heterosis occurring in hybrids of provenance crossings of different latitude in *Populus tremula*. Silvae Genetica 5: 156—160 (1956). — MCWILLIAM, J. R., and B. GRIFFING: Temperature-dependent heterosis in maize. Aust. J. Biol. Sci. 18: 569—583 (1965). — NILSSON, B. O.: Intraspecific hybridization and heterosis within *Picea abies*. Food and Agriculture Organization of the United Nations. FAO/FORGEN 2b/6: 1—9, 1963. — ORR-EWING, A. L.: Inter- and intraspecific crossing in Douglas-fir, *Pseudotsuga menziesii* (MIRB.) FRANCO. Silvae Genetica 15: 121—126 (1966). — WELLS, O. O., and PHILIP C. WAKELEY: Geographic variation in survival, growth and fusiform-rust infection of planted loblolly pine. Forest Science Monograph 11: 1—40, 1966.

Embryo Development and Hybridity Barriers in the White Pines (Section Strobus)

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The study of ovule histogenesis in a non-crossable or weakly crossable species combination establishes the time of breakdown in the developmental sequence. It also indicates the general nature of the barrier and may suggest possible ways of overcoming it or of increasing the yield of difficult crosses. In addition, it may provide new information on taxonomic affinities to supplement that obtained from crossability patterns.

Seed failure in pines can occur before, during, or after fertilization (BUCHHOLZ 1944). In the hard pines (subgenus *Pinus [Diploxylon]*)[1]), several workers have reported that hybrid failure occurred at an early stage because of the inability of pollen tubes to function normally in the nucellar tissue of the foreign species. The incompatibility is presumed to be the result of chemical differences limiting pollen tube growth in the female gametophyte. Some possible factors involved are amino acids (MCWILLIAM 1959), auxin inhibitors (HASHIZUME and KONDO 1962 a, 1962 b), and sugars (CHIRA and BERTA 1965). In some crosses, low-level gamma irradiation of the pollen may stimulate subsequent pollen tube growth and overcome inhibition (VIDAKOVIĆ 1963).

There is no evidence of pollen tube incompatibility in the soft or white pines (subgenus *Strobus [Haploxylon]*). In this group, any early seed failures appear to be associated with a lack of pollination, inherent characteristics of the seed tree, or other causes not attributable to the species cross (KRIEBEL 1967). Development of the gametophytes during the first year after pollination is normal regardless of species combination (UEDA *et al.* 1961). The process is identical to that of *P. strobus* L. as described by FERGUSON (1904).

The sterile crosses *P. peuce* GRISEB. × *P. cembra* L. and *P. peuce* × *P. koraiensis* SIEB et ZUCC. were studied by HAGMAN and MIKKOLA (1963). In each cross the parents are in different subsections of section *Strobus*. No crosses between species of subsection *Cembrae* and those of subsection *Strobi* have been successful, except those involving *P. lambertiana* DOUGL., which may have been erroneously placed in Strobi (WRIGHT 1962). HAGMAN and MIKKOLA found that fertilization took place in ovules of *P. peuce* × *cembra* and in most ovules of *P. peuce* × *koraiensis*. Degeneration occurred inside the archegonia at the proembryo stage as defined by DOYLE (1963).

Initial work on *P. strobus* × *cembra* and *P. strobus* × *koraiensis* indicated that the developmental pattern was similar to that found in the *P. peuce* hybrids, though some ovules contained the earliest stages of a true embryo (KRIEBEL 1967, 1968). Embryogeny followed the typical pattern described by BUCHHOLZ (1918, 1929, 1931) up to the time of abortion. This report includes subsequent research and presents quantitative data on comparative embryogeny in hybrid ovules of viable and inviable species combinations.

[1]) Terminology of CRITCHFIELD and LITTLE (1966) and SHAW (1914), respectively; that of CRITCHFIELD and LITTLE is used in this paper.

Materials and Methods

Controlled pollinations were made on *P. strobus* in each of 3 years. The male parents included *P. strobus* as a control, one weakly crossable species (*P. flexilis* JAMES)[2]), and 2 species not crossable with *P. strobus* (*P. cembra, P. koraiensis*). Whenever possible, more than one male and female parent was used for each species cross. With the exception of *P. koraiensis*, each of the species used as a pollen parent was included in 2 or more years' experiments. Pollens of *P. strobus* and *P. flexilis* were freshly collected; those of other species had been stored at —12° C for 1 to 4 years.

Immature female strobili were collected during the second growing season. We had previously established that the critical period of archegonial development, fertilization, and embryo formation occurred in the last half of June in the experimental area. Therefore, the 1966 collections were made daily during the period 16 June 1966 to 30 June 1966 and on 3 dates in July and August. In the second experiment, collections were made daily from 18 June 1968 to 3 July 1968 and on 5 later dates in July. The 1969 collections, again daily, covered the period 26 June to 3 July.

The immature strobili were collected in the early morning and brought to the laboratory within 1 hour of the time of picking. The cones were weighed and measured, then dissected. The ovules were fixed in Craf V (SASS 1951) in a vacuum desiccator and kept in cold storage at 4° C. They were dehydrated with TBA (JOHANSEN 1940) and embedded in Tissuemat or Paraplast.

Each block contained 8 ovules, with occasional exceptions. The blocks were cut into 13 μ serial sections on a rotary microtome. Usually, 3 sections were mounted on a slide and 28 to 36 slides were made from a block of 8 ovules. A part of the first year's slides were stained with safranin and anilin blue; subsequently all slides were prepared with JOHANSEN's quadruple stain (JOHANSEN 1940).

[2]) The size (38 ft. height at age 56), needle and cone characteristics verify the species as *P. flexilis* JAMES, not *P. strobiformis* ENGELM.