Arct. Alp. Res. **3**: 277—289 (1971). —— Blanchet, B.: Les cedrieres de Quebec. Etudes Ecologiques No. 6. M. M. Grandtner (Ed). Laboratoire d'ecologie forestiere, Universite Laval, Quebec (1982). —— Bousquet, J., Cheliak, W. M. and Lalonde, M.: Genetic differentiation among 22 mature populations of green alder (*Alnus crispa*) in central Quebec. Can. J. For. Res. **17**: 219—227 (1987). —— Briggs, D. and Walters, S. M.: Plant variation and evolution. Second edition. Cambridge University Press, Cambridge (1984). —— Caulkins, H. L.: The ecology and reproduction of Northern White Cedar. Master of Forestry Thesis, The University of Michigan, Ann Arbor (1967). —— Chakraborty, R.: Gene-diversity analysis in nested subdivided populations. Genetics **96**: 721—723 (1980). —— Cheliak, W. M., Wang, J. and Pitel, J. A.: Population structure and genic diversity in tamarack, *Larix laricina* (Du Roi) K. Koch. Can. J. For. Res. **18**: 1318—1324 (1988). —— Copes, D. L.: Isoenzyme uniformity in western red cedar seedlings from Oregon and Washington. Can. J. For. Res. **11**: 451—453 (1981). —— Crow, J. F.: Basic concepts in population, quantitative, and evolutionary genetics. W. H. Freeman and Co., New York, Oxford (1986). —— Davis, P. H. and Heywood, V. H.: Principles of angiosperm taxonomy. D. Van Nostrand Co., Princeton, N. J., New York (1967). —— El-Kassaby, Y. A. and Sziklai, O.: Genetic variation of allozyme and quantitative traits in a selected Douglas-fir (*Pseudotsuga menziesii* var. menziesii (Mirb.) Franco) population. For. Ecol. Manage. **4**: 115—126 (1982). —— Fins, L. and Seeb, L. W.: Genetic variation in allozymes of western larch. Can. J. For. Res. **16**: 1013—1018 (1986). —— Fowler, D. P. and Morris, R. W.: Genetic diversity in red pine: evidence for low genic heterozygosity. Can. J. For. Res. **7**: 343—347 (1977). —— Gottlieb, L. D.: Enzyme differentiation and phylogeny in *Clarkia franciscana, C. rubicunda*, and *C. amoena*. Evolution **27**: 205—214 (1973). —— Grant, M. C. and Mitton, J. B.: Genetic differentiation among growth forms of Engelmann spruce and subalpine fir at tree line. Arct. Alp. Res. **9**: 259—263 (1977). —— Gregor, J. W.: The ecotype. Biol. Rev. **19**: 20—30 (1944). —— Habeck, J. R.: White cedar ecotypes in Wisconsin. Ecology **39**: 457—463 (1958). —— Hamrick, J. L., Linhart, Y. B. and Mitton, J B.: Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. **10**: 173—200 (1979). —— Harris, H. and Hopkinson, D. A.: Handbook of enzyme electrophoresis in human genetics. Elsevier, North-Holland Publ. Co., Amsterdam (1976). —— Hiebert, R. D. and Hamrick, J. L.: Patterns and levels of genetic variation in Great Basin bristlecone pine, *Pinus longaeva*. Evolution **37**: 302—310 (1983). —— Krebs, C. J.: Ecology: The experimental analysis of distribution and abundance. Harper and Row, New York (1972). —— Larson, D. W.: Effects of disturbance on old-growth *Thuja occidentalis* at cliff edges. Can. J. Bot. **68**: 1147—1155 (1990). —— Linhart, Y. B., Mitton, J. B., Sturgeon, K. B. and Davis, M. L.: Genetic variation in space and time in a population of ponderosa pine. Heredity **46**: 407—426 (1981). —— Loveless, M. D. and Hamrick, J. L.: Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. **15**: 65—95 (1984). —— Merkle, S. A. and Adams, W. T.: Patterns of allozyme variation within and among Douglas-fir breeding zones in southwest Oregon. Can. J. For. Res. **17**: 402—407 (1987). —— Moran, G. F. and Adams, W. T.: Microgeographical patterns of allozyme differentiation in Douglas-fir from southwest Oregon. Forest Sci. **35**: 3—15 (1989). —— Musselman, R. C., Lester, D. T. and Adams, M. S.: Localized ecotypes of *Thuja occidentalis* in Wisconsin. Ecology **56**: 647—655 (1975). —— Nei, M.: Genetic distance between populations. Am. Nat. **106**: 283—292 (1972). —— Nei, M.: Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA **70**: 3321—3323 (1973). —— Nei, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583—590 (1978). —— O'Reilly, G. J., Parker, W. H. and Cheliak, W. M.: Isozyme differentiation of upland and lowland *Picea mariana* stands in northern Ontario. Silvae Genetica **34**: 214—221 (1985). —— Perry, D. J. and Knowles, P.: Inheritance and linkage relationships of allozymes of eastern white cedar (*Thuja occidentalis*) in northwestern Ontario. Genome **32**: 245—250 (1989). —— Perry, D. J. and Knowles, P.: Evidence of high self-fertilization in natural populations of eastern white cedar (*Thuja occidentalis*). Can. J. Bot. **68**: 663—668 (1990). —— Perry, D. J., Knowles, P. and Yeh, F. C.: Allozyme variation of *Thuja occidentalis* L. in northwestern Ontario. Biochem. Syst. Ecol. **18**: 111—115 (1990) —— Plessas, M. E. and Strauss, S. H.: Allozyme differentiation among populations, stands, and cohorts in Monterey pine. Can. J. For. Res. **16**: 1115—1164 (1986). —— Potzger, J. E.: Vegetative reproduction in conifers. Am Midl. Nat. **18**: 1001—1004 (1937). —— Roose, M. L. and Gottlieb, L. D.: Genetic and biochemical consequences of polyploidy in *Tragopogon*. Evolution **30**: 818—830 (1976). —— Shaw, C. R. and Prasad, R.: Starch gel electrophoresis of enzymes: a compilation of recipes. Biochem. Genet. **4**: 279—320 (1970). —— Simon, J.-P., Bergeron, Y., and Gagnon, D.: Isozyme uniformity in populations of red pine (*Pinus resinosa*) in the Abitibi region, Quebec. Can. J. For. Res. **16**: 1133—1135 (1986). —— Spurr, S. H. and Barnes, B. V.: Forest Ecology. Third Edition. John Wiley and Sons, New York (1980). —— Squillace, A. E. and Bingham, R. T.: Localized ecotopic variation in western white pine. Forest Sci. **4**: 20—34 (1958). —— Stutz, H. P. and Mitton, J. B.: Genetic variation in Engelmann spruce associated with variation in soil moisture. Arct. Alp. Res. **20**: 461—465 (1988). —— Teich, A. H. and Holst, M. J.: White spruce limestone ecotypes. For. Chrom. **50**: 110—111 (1974). —— Turesson, G.: The species and varieties as ecological units. Hereditas **3**: 100—113 (1922a). —— Turesson, G.: The genotypical response of the plant species to the habitat. Hereditas **3**: 211—350 (1922b). —— Unterscheutz, P., Ruetz, W. F., Geppert, R. R., and Ferrell, W. K.: The effect of age, preconditioning, and water stress on the transpiration rates of douglas fir (*Pseudotsuga menziesii*) seedlings of several ecotypes. Physiol. Plant. **32**: 214—221 (1974). —— Wells, O. O.: Geographic variation in ponderosa pine. I. The ecotypes and their distribution. Silvae Genetica **13**: 89—103 (1964). —— Wright, S.: Evolution in Mendelian populations. Genetics **6**: 97—159 (1931). —— Yeh, F. C.: Isozyme variation of *Thuja plicata* (Cupressaceae) in British Columbia. Biochem. System. Ecol. **16**: 373—377 (1988). —— Yeh, F. C. and Layton, C.: The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* ssp. latifolia. Can. J. Genet. Cytol. **21**: 487—503 (1979). —— Yeh, F. C. and O'Malley, D.: Enzyme variations in natural populations of douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, from British Columbia. 1. Genetic variation patterns in coastal populations. Silvae Genetica **29**: 83—92 (1980). —— Zavitkovsky, J. and Ferrell, W. K.: Effect of drought upon rates of photosynthesis, respiration and transpiration of seedlings of two ecotypes of Douglas-fir. Bot. Gaz. **129**: 346—350 (1968).

Geographic Variation in Mississippi Loblolly Pine and Sweetgum

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Summary

Seed was collected along latitudinal transects from 575 loblolly pine (*Pinus taeda* L.) and 650 sweetgum trees (*Liquidambar styraciflua* L.) distributed throughout Mississippi and adjacent parts of neighboring states. Progenies were compared on the basis of various morphological and phenological traits both in the nursery and after 10 years in plantings in the southern, central, and northern parts of the study area. Patterns of variation are presented graphically on a series of maps.

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This paper is dedicated to the memory of Dr. Switzer.

Variation in both species has apparently evolved in response to the selection forces exerted by latitude-related (climatic) variables. Both species, however, show longitude-related variation in several traits which can be explained by assuming that both species were isolated into eastern and western populations during the Pleistocene.

Key words: genecology, provenance-progeny test, clinal patterns, sympatric species, Pinus taeda L., *Liquidambar styraciflua* L., climatic variables.

Introduction

Loblolly pine (*Pinus taeda L.*) and sweetgum *(Liquidambar styraciflua* L.) are widely distributed and commercially important forest species of the southeastern United States (*Fig. 1*). The two are common associates throughout the range of loblolly pine but sweetgum ranges farther north and inland than does the pine. Comparing patterns of geographic and tree-to-tree variation in the two species and relating differences and similarities to factors that could have affected their genetic structure can lead to a greater understanding of the evolution of the two species.

Both species are prolific seeders, competing with each other to colonize open land in the southeastern United States. Sweetgum sprouts vigorously, adding to its reproductive ability, whereas the pine does not. Sweetgum occupies most of the same ecological niches as loblolly pine but is less resistant to fire and, in the absence of fire, becomes abundant on pine sites.

A major difference of adaptive and evolutionary significance between the two species is that loblolly pine is not widely distributed on the floodplain of the Mississippi River whereas sweetgum is. This difference in distribution has probably had an effect on the comparative genetic structure of the two species because the floodplain divides the range of loblolly pine into two reproductively isolated populations whereas the sweetgum range is continuous across the floodplain. None of the other rivers in the southeastern Coastal Plain are known to present a major obstacle to migrating pollen and seed of loblolly pine, but the Mississippi River floodplain has a pineless exposure that is 30 km to 50 km wide at its narrowest point near Vicksburg, and about 240 km wide near Greenville, Mississippi.

Several factors retard the formation of local ecotypes in both species. They are both monecious and wind pollinated, and the seed of both is light, winged, and widely distributed. Both begin to produce seed in abundance at 20 to 30 years of age and thus the turnover of sexual generations is probably similar. When sweetgum reproduces by sprouting, an asexual generation may persist indefinitely. Reproduction of both species is primarily by outcrossing in nature, and loblolly pine, at least, has mechanisms to prevent self-pollination (Kraus and Squillace, 1964). Strobili occur primarily in the upper portion of the pine tree crowns while pollen is produced in the middle and lower parts. Selfs of loblolly pine are generally less vigorous than outcrossed progeny and therefore do not compete well (Franklin, 1969). The degree of inbreeding has not been determined for sweetgum, but both species are probably highly heterozygous.

The evolutionary history of the two species has probably been very different until comparatively recent geologic time. Scattered, isolated populations of sweetgum that occur in the highlands of Central America are separated from the main area of the distribution by 800 miles of grassland and desert in northeastern Mexico and southern Texas. Presumably, sweetgum bridged this present void at some point in geologic time but climatic conditions have not been favorable for such continuity since the Pleistocene or perhaps as long ago as the Pliocene (Delcourt and Delcourt, 1981). The only member of the Pinaceae with a disjunct range including the United States and Central America is *Pinus strobus* L., a five needled white pine.

Whether or not loblolly pine ever occurred in Central America is unknown. Other hard pines do occur in Central America, however, and recent work with chloroplast DNA suggests that loblolly pine may be much more closely related to Chihuahua pine (*P. leiophylla* Schiede and Deppe) than has previously been supposed (Strauss et al., 1989). Chihuahua pine has a wide-spread distribution in the mountains of Mexico.

Since some unknown time prior to the Holocene, a major difference of evolutionary significance between the two species has been the aforementioned division of loblolly into two reproductively isolated populations by the floodplain of the Mississippi River.

Another important difference between the species with undoubted evolutionary significance is the association of related, sympatric species. Sweetgum is the only *Liquidambar* species in North America but loblolly pine has at least five other hard pines of subsection Australes, within or near its present range, with which it could exchange genes: Shortleaf pine (*Pinus echinata* Mill.) occurs throughout most of the loblolly range; slash (*P. elliottii* Engelm. var. *elliottii*) and longleaf (*P. palustris* Mill.) pines are sympatric with loblolly in the south; and, in the northeast, loblolly impinges on the range of pond (*P. serotina* Michx.) and pitch (*P. rigida* Mill.) pines. Crossability studies have shown all of these species to be potentially interfertile, and gene exchange in nature has been demonstrated between loblolly and all of them except slash pine (Smouse and Saylor, 1973). In past geologic time, most of the *Diploxylon* pines in North America could have exchanged genes, but the other species of *Liquidambar* are Asiatic so any gene exchange among them would have had to take place much earlier than in North American pines.

The climatic environment within which both species presently grow is quite uniform over long distances, particularly in the Coastal Plain and the Piedmont physiographic provinces. The environment of the uplands between streams is fairly uniform at a given latitude from the Mississippi River to the Atlantic Coast, at least in terms of selection pressures that would presumably affect sweetgum or loblolly pine. Gene flow within these areas is determined primarily by temperature, precipitation, and, perhaps, day length. The effects of genetic drift are probably minimal under these circumstances.

Evolutionary effects of soil variability within these areas is probably minimal. Evidence of adaptive ecotypes has been scant in both species in spite of extensive testing (particularly with loblolly pine) done in conjunction with tree improvement programs.

Given this array of similarities and differences, the hypothesis tested was whether or not the two species have evolved different patterns of variation. Further, if their patterns of variation are different in some respect, are these differences correlated with corresponding dif-

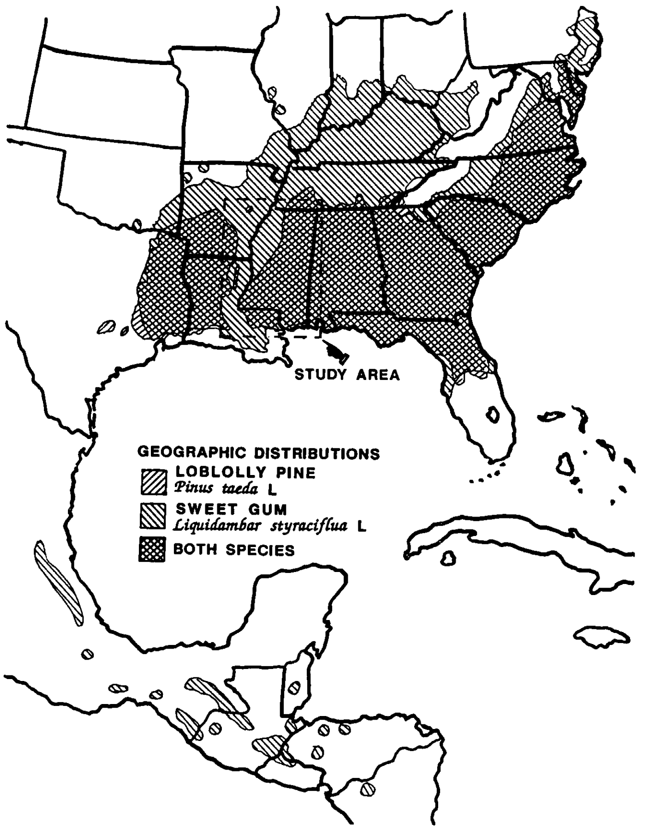


Figure 1. — Natural range of loblolly pine and sweet gum (adapted from USDA-Forest Service, 1965).

ferences in certain environmental or evolutionary determinants? This study assays the amount and pattern of genetic variation in these two sympatric species in a circumscribed portion of their range — namely, in and near the periphery of the state of Mississippi and southeastern Louisiana.

Methods

Study area

The major part of the study area is bounded on the west by the Mississippi River drainage, on the east by the drainage of the Tombigbee and Alabama Rivers, on the south by the Gulf of Mexico and on the north by the northern boundary of Mississippi (*Fig. 1*). The climate over this area is characterized as subtropical. During summer, warm, moist, maritime air predominantes, whereas in winter, cold, dry masses of arctic air occasionally penetrate. Mean annual precipitation varies from about 1626 mm along the Gulf Coast to 1270 mm in the northwest, east central, and lower Delta parts in Mississippi. Mean annual temperature ranges from 20 °C to 17.7 °C and frost-free season from 300 days to 210 days.

All of these climatic patterns have minor perturbations superimposed upon a basic north-south pattern. Mean annual precipitation (almost entirely in the form of rain) decreases rapidly with distance from 1626 mm on the Gulf Coast to 1372 mm over the southern third of the state, but then varies in an apparently random pattern between 1372 mm and 1270 mm over the rest of the state (*Fig. 2a*). There is a very small area of slightly decreased rainfall that extends from the central Gulf Coast inland 30 km to 50 km. This "drier" area does not extend east or west into Alabama or the Florida Parishes of Louisiana.

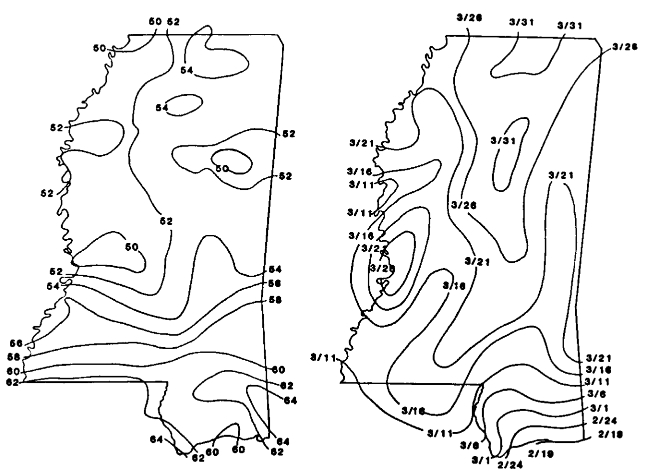
The major deviation from the basic north-south pattern for temperature is the predominance of slightly colder temperatures along a diagonal line from northeast to southwest Mississippi. This can best be seen in the date of onset of frost-free season (*Fig. 2b*). This anomaly is associated with the areas relatively high elevation that occur along the same northeast-southwest diagonal line. Elevations reach a maximum of between 180 m and 220 m in several places along this line.

The greatest elevational contrast over a short distance in the study area is between the floodplain of the Mississippi River, which is still below 60 m at its northernmost point in the state, and the above-mentioned highlands between 180 m and 220 m which begin only about 80 km east of the floodplain in the northern part of the state.

The forest of the southern half of the study area is dominated by broad expanses of pine; longleaf-slash in the east and loblolly-shortleaf in the west with a dividing corridor of oak-pine along the axis of the Pearl River, which forms the border between Louisiana and Mississippi near the Gulf Coast. The loblolly-shortleaf type terminates in the west with a transition to the narrower bands of oak-dominated types found on the loess covered uplands. The distribution of forest types in the northern half of the study area is more of a mosaic whose matrix is characterized by the region's cultivated areas. The loblolly-shortleaf type dominates the forest and extends generally northward from its southeastern occurrence to terminate at about 34 °N latitude. North of this latitude, loblolly is less common and shortleaf becomes the dominant pine. With the exception of the Tombigbee River lowlands, loblolly pine is rare in all the area's remaining forest types. In contrast to this dissected distribution of loblolly pine, sweetgum is ubiquitous throughout the entire study area.

Plant material

Seed was collected by dividing the study area east of the Mississippi River into 20 latitudinal transects, 15 minutes of latitude apart, and designating a collection point every 50 km along the 20 transects. Six loblolly pine and two sweetgum locations were also sampled west of the Missisippi River. Collections were made from natural



A. Precipitation **B. Spring freeze**

Figure 2. — Maps of Mississippi showing: A. Mean annual precipitation (in inches, one inch = 25.4 mm), and B. Last spring date with a temperature of 0 °C or lower, 1921 to 1950 average (from McWhorter, 1962).

Table 1. — Locations of plantings.

Planting	Sweetgum	Loblolly pine
Southern	Hancock County, Mississippi 30° 20′ N. Lat., 89° 30′ W. Long.	Livingston Parish, Louisiana 30° 30′ N. Lat., 90° 45′ W. Long
Central	Oktibbeha County, Mississippi 33° 30′ N. Lat., 89° 00′ W. Long.	Winston County, Mississippi 33° 30′ N. Lat., 89° 00′ W. Long
Northern	Fayette County, Tennessee 35° 30′ N. Lat., 89° 00′ W. Long.	Franklin County, Alabama 34° 30′ N. Lat., 88° 00′ W. Long

stands within 16 km of these points. Plantings, or trees within sight of plantings, were avoided. Fruits and cones were collected in 1962, 1963 ,and 1964 from five trees in each of a total of 138 sweetgum and 115 loblolly pine stands. Criteria for selecting parent trees were undemanding. The first five fruiting trees of any age to be sighted within a collection location were chosen if they were between 60 m and 400 m apart and were within 300 m of other fruiting trees of the same species. The progeny of each of the five trees from a single collection (open-pollinated families) were kept separate throughout the study.

Planting stock of each species was grown during 1965 in a three-replicate design in a nursery near Gulfport, Mississippi. Fusiform rust was prevented in the nursery by periodic applications of fungicide to the pine. The one-year-old seedlings were planted during the winter of 1965 to 1966 on locations selected to represent the southern, central and northern regions of the study area (*Table 1*).

Stock for each planting was from one nursery replication. The design of the field planting was a compact family block (WRIGHT, 1976), which is essentially a split-plot design. The main plots consisted of 10 trees from a single collection point planted in a row. Within each main plot there were five subplots (two adjacent trees) representing the five single families from a collection point. The design was replicated 10 times at each planting location. Spacing was 2.44 m x 2.44 m and each replication was about ½ hectare. A border row was used to offset edge effects.

Sweetgum Plantings

The central and northern sweetgum plantings were on bottomland old fields that had been abandoned for many years and were occupied mostly with grass. The southern planting of sweetgum was on a low sandy ridge adjacent to marshland along the north coast of Lake Borgne that was recently cleared forest land (loblolly pine and mixed hardwoods, including sweetgum). The topsoil was shallow, and much of it was lost during site preparation.

Grasses were partially cotrolled in all sweetgum plantings the first year by mowing and application of herbicide around each tree. Growth in these plantings was slow in the early years (trees were less than 1.7 m tall after 4 years), but generally rapid thereafter. This delay in initiation of rapid height growth probably added experimental error to the experiment, for genetic differences in height among families and populations are to some extent attributable to differences in time of initiation of height growth rather than straightforward differences in growth rate. Because of continued poor growth, three replicates were discarded in the northern and southern plantings and two in the central planting (*Table 2*). Survival averaged 83.6 % at 10 years in the remaining, usable replicates.

Table 2. — Characteristics of the sweetgum plantings after ten years.

Planting	Mean height	Replicates	Total collection points	Collection points having five families	four families	three families
	(m)			number		
Southern	2.46	7	138	125	10	3
Central	4.20	8	136	125	7	4
Northern	4.23	7	133	118	9	6

Loblolly plantings

Early survival was excellent in the southern planting, which was on recently cleared forest land, but poor in the central and northern plantings. In the central planting, which was on an old agricultural field, eight of the 10 replicates were killed by a hard freeze the day after planting and only the two that were planted a few days later survived. In the northern planting, mortality was extensive in seven replicates planted on an eroded pasture, but three replicates on recently cleared forest land survived well. Growth was excellent in the surviving replicates (*Table 3*). In all plantings of both species, mortality reduced some collection points to representation by less than three families; these were excluded from the analysis.

Table 3. — Characteristics of pine plantings after 10 years.

Planting	Mean height	Fusiform rust infection: Stem & branch	Stem	Usable replicates	Survival in usable replicates	Collection points having five families	four families	three families
	M	%		Number	%	Number		
Southern	10.23	44.3	39.9	10	66.9	99	8	6
Central	9.99	74.6	29.9	2	57.9	37	28	33
Northern	8.91	17.4	7.7	3	52.6	34	24	17

Measurement and analysis

Variation in one or both of the two species was measured for height through the tenth year, crown form, fusiform rust infection, drought resistance, and timing of leaf shed in the fall. These traits were measured in the progeny — either in the nursery or field plantings or both. Seed weight of the parent trees was also measured. The means across all the families from a single collection point — the source means, are presented as a positive (▲) or negative (▽) triangles representing values above or below the grand mean of all the collection points of a map.

All data except seed weights were subjected to analysis of variance. ANOVA was first used to determine any differences among families from a given seed collection area when grown at a particular planting location. The experimental unit was the mean of the two-tree family plots. Values were calculated for missing plots and adjustments made to the treatment sums of squares and degrees of freedom for error (YATES, 1933); augmented values were used thereafter when error and family sums of squares were pooled for analyses of variance for data from an entire planting. When ANOVA indicated significant difference for a trait among collection point means

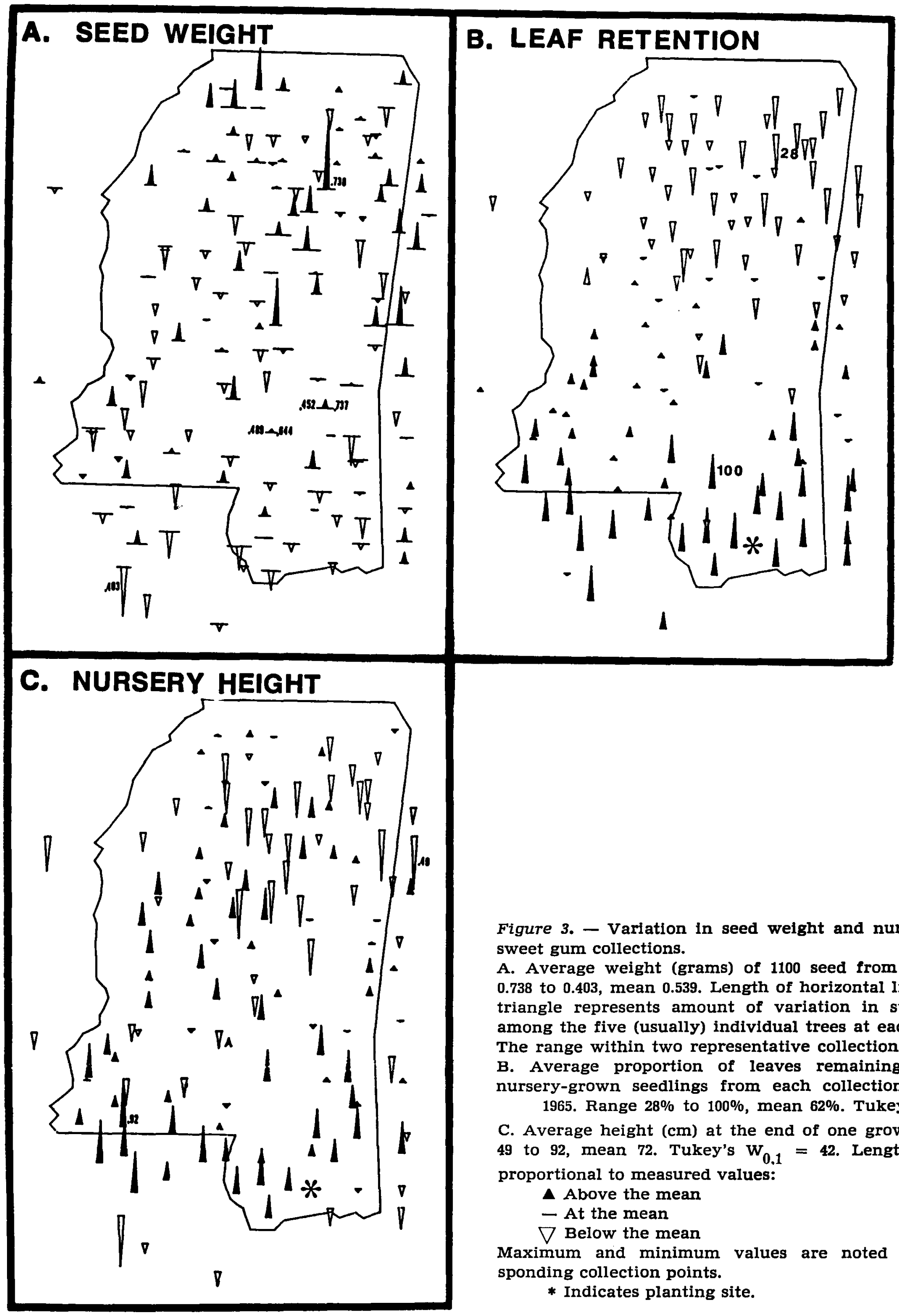


Figure 3. — Variation in seed weight and nursery characters of sweet gum collections.

A. Average weight (grams) of 1100 seed from each tree. Range, 0.738 to 0.403, mean 0.539. Length of horizontal line at base of each triangle represents amount of variation in standard deviations among the five (usually) individual trees at each collection point. The range within two representative collection points is shown.

B. Average proportion of leaves remaining on one-year-old nursery-grown seedlings from each collection on 21 December 1965. Range 28% to 100%, mean 62%. Tukey's $W_{0.1}$ = 42.

C. Average height (cm) at the end of one growing season. Range 49 to 92, mean 72. Tukey's $W_{0.1}$ = 42. Length of triangles are proportional to measured values:

▲ Above the mean

— At the mean

▽ Below the mean

Maximum and minimum values are noted beside the corresponding collection points.

* Indicates planting site.

in a particular planting, Tukey's (1951) multiple range test was conducted and results presented on the applicable map.

Sweetgum Results

Seed weight

In general, collections from the northern half to the study area had heavier seed than those from the southern half (*Fig. 3a*). However, the pattern was not a smooth north-south cline, for a large area of sources with particularly heavy seed exists in the east-central part of the state, and some sources in the extreme northwestern corner were also heavy-seeded. Sources from the floodplain of the Mississippi River generally had lighter seed than those from the same latitude farther east. The lightest seed of all originated from south of New Orleans. Genetics effects cannot be separated from environmental influences in this trait as they can for traits assayed in the "common garden" part of the experiment. However, seed weight is important for its covariate effects on other traits, such as early height growth. where phenotypic and genotypic effects can be separated.

Wilcox (1968), using a subsample of this sweetgum seed, reported that the requirement for cool, moist stratifica-

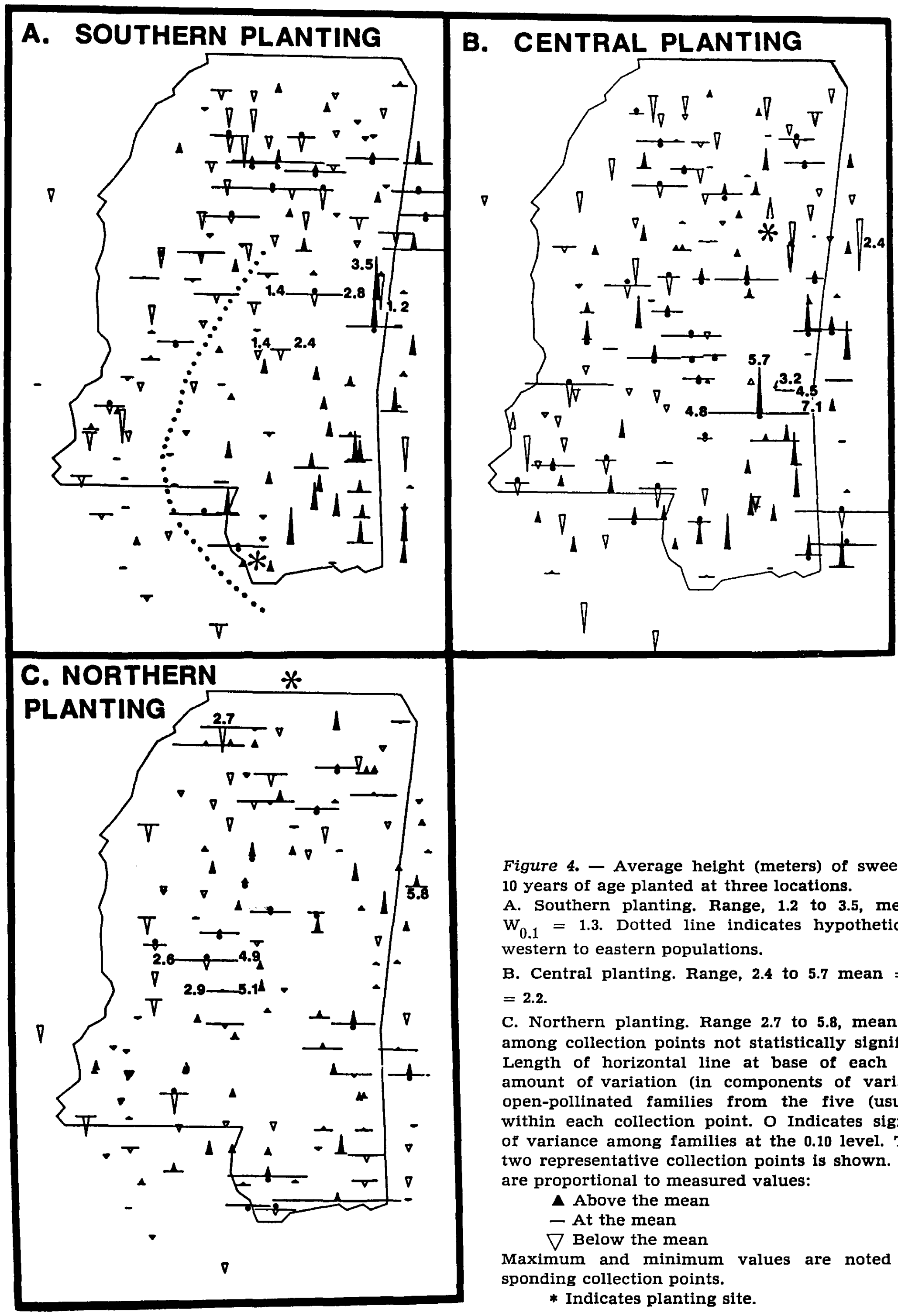


Figure 4. — Average height (meters) of sweetgum collections at 10 years of age planted at three locations.
A. Southern planting. Range, 1.2 to 3.5, mean = 2.3. Tukey's $W_{0.1}$ = 1.3. Dotted line indicates hypothetical transition from western to eastern populations.
B. Central planting. Range, 2.4 to 5.7 mean = 4.2. Tukey's $W_{0.1}$ = 2.2.
C. Northern planting. Range 2.7 to 5.8, mean = 4.4. Differences among collection points not statistically significant.
Length of horizontal line at base of each triangle represents amount of variation (in components of variance units) among open-pollinated families from the five (usually) parent trees within each collection point. O Indicates significant component of variance among families at the 0.10 level. The families within two representative collection points is shown. Length of triangles are proportional to measured values:
▲ Above the mean
— At the mean
▽ Below the mean
Maximum and minimum values are noted beside the corresponding collection points.
* Indicates planting site.

tion increased with increasing latitude of seed origin. The seed originating near the mouth of the Mississippi River required the shortest period of stratification while that from the northern part of the study area required the longest.

Leaf retention in fall

This trait is obviously under very strong genetic control (*Fig. 3b*). Trees of northern origin lose their leaves earlier in the fall than do trees from farther south; the pattern of variation is a fairly smooth cline. Genetic control is so strong that differences in response between sources from similar latitudes but different elevations are discernible — e. g., the difference between northwestern sources from the Mississippi River floodplain (30 m to 60 m) and the uplands of the northeast (maximum elevation 250 m). Furthermore, no within-stand variation was discernible in this trait.

Height

After a growing season in the nursery, height did not show as strong a pattern of variation as did leaf retention, but the patterns of the two traits were quite similar. It was evident that trees from the southern part of the study area were taller than those originating in northern Mississippi (*Fig. 3c*). However, trees from south of New Orleans, and the southeastern Mississippi coast were markedly shorter than others of southern origin. There were also areas in northern, southwestern, and southern Mississippi where growth was much below average.

Height at 10 years in the southern planting indicates a strong tendency for sweetgum sources from the southeast and east-central to excel (*Fig. 4a*). This tendency was also fairly well expressed in the central planting (*Fig. 4b*) but was less evident in the northern planting (*Fig. 4c*). The southeastern trees evidently have the genetic potential for relatively fast growth but when moved north, first to the central and then to the northern planting locations, fewer and fewer of them express this potential. Presumably if a fourth, still more northern planting had been included, the zone of optimum growth would have migrated northwest to the latitude of central or northern Mississippi.

The latitudinal effects found with sweetgum have also been found in most other wide-ranging tree species that have been intensively tested and are apparently a common influence in the evolution of growth rate differences in tree species. However, the longitudinal aspects of the variation pattern are quite unexpected. For any given latitude, sweetgum from the western part of the study area grew more slowly than those from the eastern half of the study area. The contrast between the eastern and western sources is especially distinct in the southern Mississippi planting but is also discernible in all three plantings. After 10 years, eastern trees in the southern planting were roughly 0.6 m taller than those from comparable latitudes of the Mississippi River floodplain. The slow growth of the Mississippi River floodplain trees is puzzling because selection for fast growth is generally greatest on optimum sites (Squillace and Kraus, 1959) and the floodplain sites are, in general, better than upland sites.

In the southern planting, occurrence of sweetgum sources with appreciable within-population variability in 10-year height appears to be patterned — i. e., there is more variability among the families within sources in the north and west than in the south. However, this is well correlated with average height of trees from these sources. The southeastern part of the study area has the tallest trees and the fewest instances of significant within-stand variability. This leads to the hypothesis that the two effects are not independent. The greatest variability among families from a single source may occur when one or more of the families were late or early in initiating rapid height growth. The effect is not as clear in the central and northern plantings (*Figs. 4b* and c), but the faster-growing trees from the southeastern part of the study area do have the least amount of within-source variability in both these plantings.

Crown form

The populations that were taller at 10 years also scored better in this quality trait (*Fig. 5*). In addition to being taller, trees from the southeastern part of the study area were straighter, more finely branched, and had a larger or more desirable ratio of height to crown width — that is, they were more columnar. These traits, while useful for choosing seed sources for forestry application (fast growth and desirable form can be found in the same selections), may not be independent. Desirable form may actually be caused by fast height growth.

Overview

Sweetgum shows variation in three independent traits: Seed weight, timing of leaf shed in the fall, and early height growth. In the study plantings, where height growth was generally very slow for about the first 5 years, variation, both between and within sources, in 10-year height was probably partly determined by differences in the initiation of rapid height growth. The east-west aspect of the variation may also be related to the initiation of rapid height growth. The pattern of variation in 1-year height in the fertile nursery environment, where there was no delay in height growth, was basically a north-south cline. Whether or not the east-west pattern would have been expressed if height growth had commenced immediately in the field plantings is not known.

Loblolly Pine

Seed weight

The seed weight of loblolly pine increased with increasing latitude and yielded a relatively smooth north-south cline (*Fig. 6a*), which agrees with the cline of decreasing precipitation (*Fig. 2a*). Three sources in extreme southwestern Mississippi had particularly light seed. The clinal pattern of this trait also agreed with the suggestion that selection pressure in drier environments is for larger seeds in many woody plants (Baker, 1972), a suggestion that also applies to the general pattern found with sweetgum seed (*Fig. 3a*). Also similar for the populations of both species

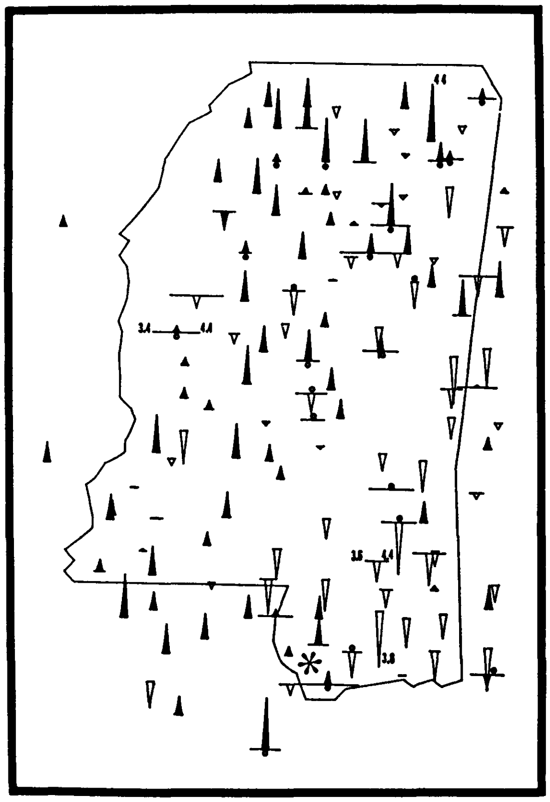


Figure 5. — **Sweet gum crown form at 10 years in the southern planting. Grade 1 = most columnar form, grade 5 = least columnar form. Range 3.75 to 4.35, mean = 4.08. Tukey's $W_{0.1}$ = 1.26 See *figure 4* for explanation of symbols.**

is the uniformity of variation within the families of a source, particularly throughout the area east of the Mississippi River. However, seed weights of loblolly pine west of the Mississippi River in the northern portion of the study pattern contradict the increases expected with the decreasing precipitation pattern. This indicates the influence of an evolutionary force other than climate-related selection pressure. Introgression with the light-seeded shortleaf pine (Hare and Switzer, 1969; Florence and Hicks, 1980) may affect seed weight in the western populations.

An intensive study of needle characters, however, failed to show any evidence of recent introgression of shortleaf genes into eastern or western populations of loblolly (Wells et al, 1977). The latitudinal band of heavier seeded sources across the southern edge of central Mississippi is another possible area of introgression — in this instance, with longleaf pine. This band corresponds to the northern limits of longleaf pine, an area where natural hybrids of longleaf and loblolly pine are of common occurrence.

The relationship between seed weight and early growth rate has been controversial in interpretation of progeny

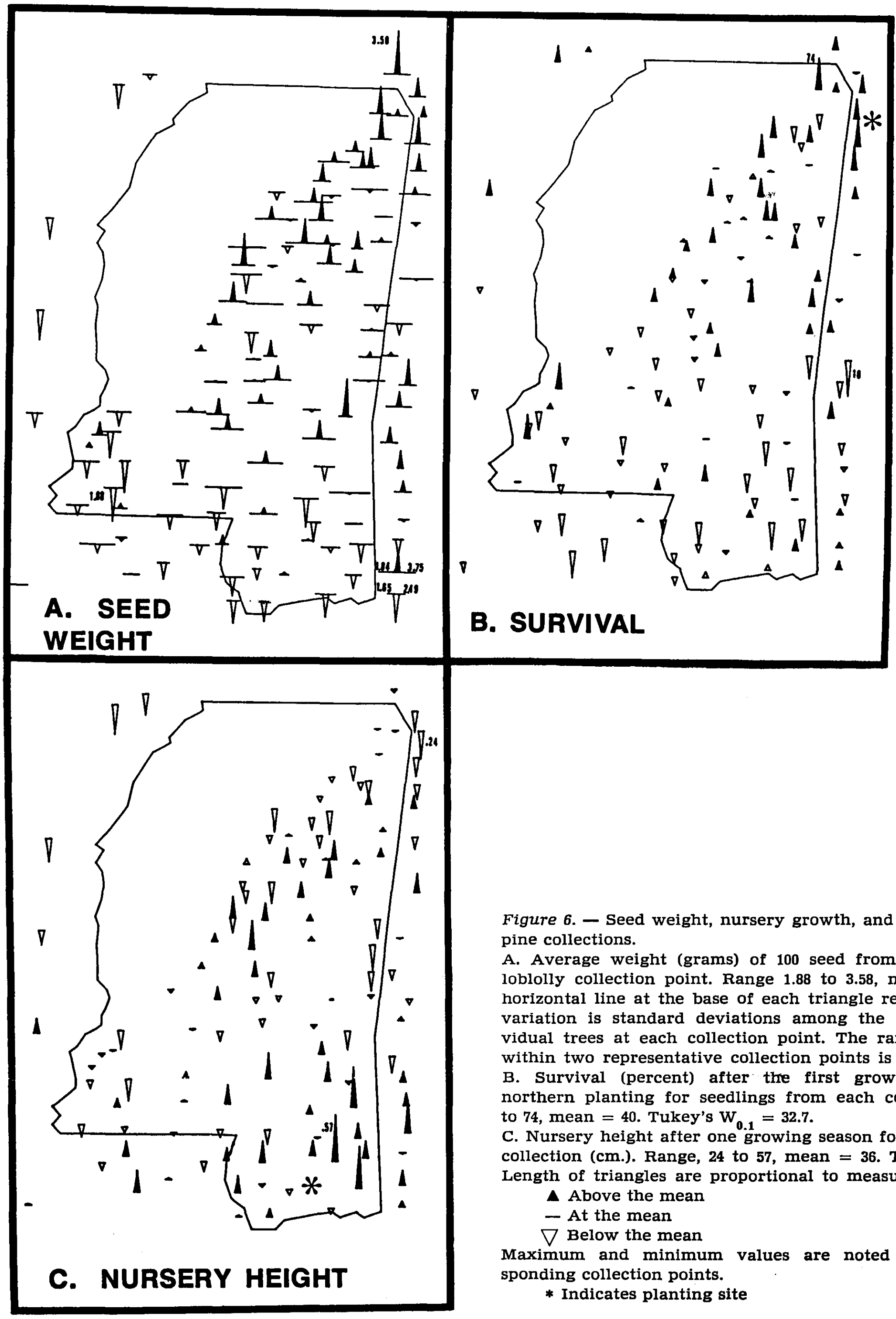


Figure 6. — Seed weight, nursery growth, and survival of loblolly pine collections.

A. Average weight (grams) of 100 seed from each tree at each loblolly collection point. Range 1.88 to 3.58, mean 2.60. length of horizontal line at the base of each triangle represents amount of variation is standard deviations among the five (usually) individual trees at each collection point. The range in seed weight within two representative collection points is shown.

B. Survival (percent) after the first growing season in the northern planting for seedlings from each collection. Range, 10 to 74, mean = 40. Tukey's $W_{0.1}$ = 32.7.

C. Nursery height after one growing season for each loblolly pine collection (cm.). Range, 24 to 57, mean = 36. Tukey's $W_{0.1}$ = 23.8. Length of triangles are proportional to measured values;

▲ Above the mean
— At the mean
▽ Below the mean

Maximum and minimum values are noted beside the corresponding collection points.

* Indicates planting site

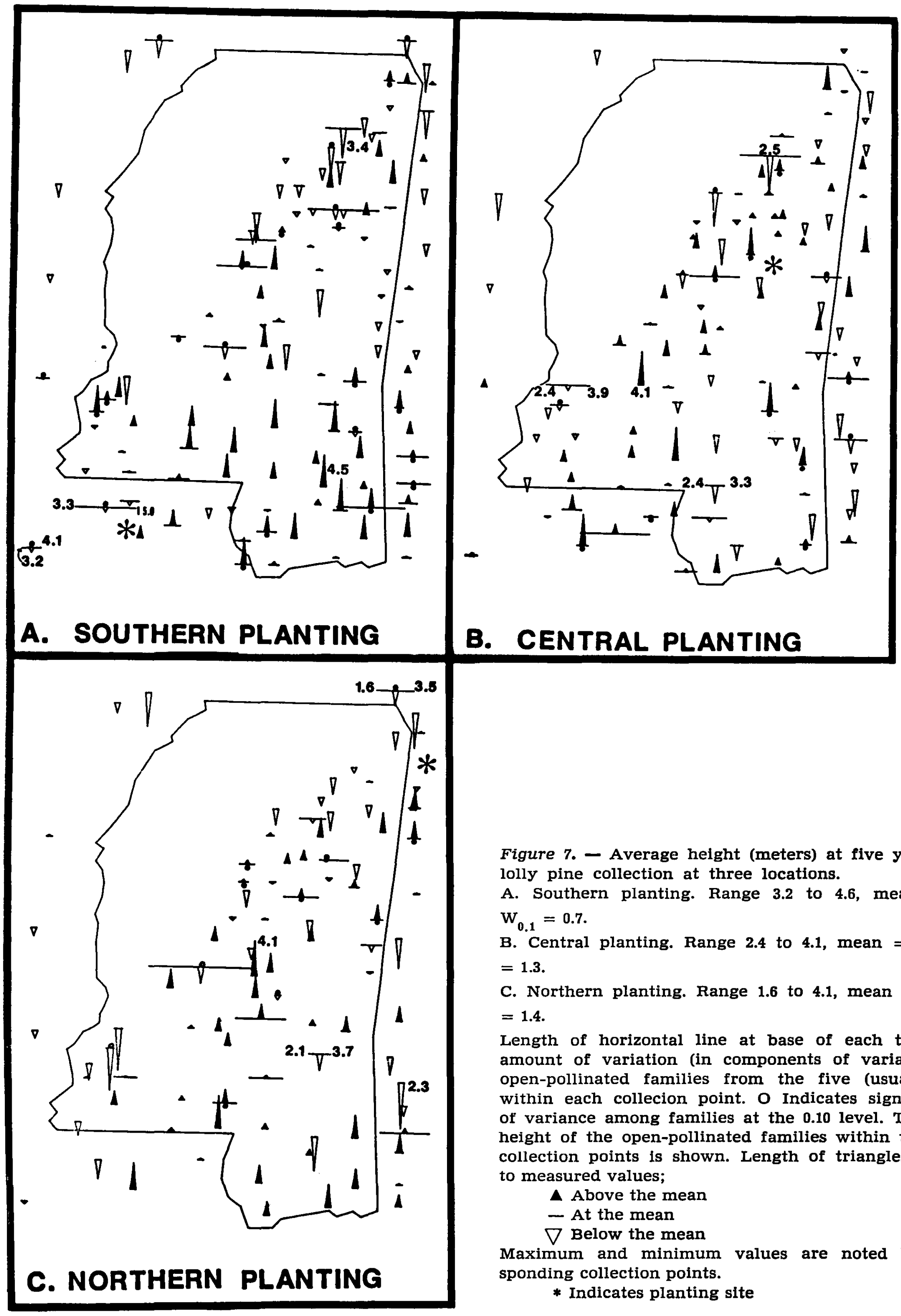


Figure 7. — Average height (meters) at five years for each loblolly pine collection at three locations.

A. Southern planting. Range 3.2 to 4.6, mean = 3.9 Tukey's $W_{0.1}$ = 0.7.

B. Central planting. Range 2.4 to 4.1, mean = 3.4 Tukey's $W_{0.1}$ = 1.3.

C. Northern planting. Range 1.6 to 4.1, mean = 3.3 Tuyey' $W_{0.1}$ = 1.4.

Length of horizontal line at base of each triangle represents amount of variation (in components of variance units) among open-pollinated families from the five (usually) parent trees within each collecion point. O Indicates significant component of variance among families at the 0.10 level. The range in mean height of the open-pollinated families within two representative collection points is shown. Length of triangles are proportional to measured values;

▲ Above the mean
— At the mean
▽ Below the mean

Maximum and minimum values are noted beside the corresponding collection points.

* Indicates planting site

test results in the southern pines and other species (Perry, 1976). In this study, the pattern of variation in height after only 1 year in the nursery and later in the out-plantings shows southern populations generally growing faster than northern ones, exactly the opposite effect expected if seed weight were the determinate (*Fig. 6c*). Obviously, the inherent potential for fast growth in trees of southern origin is a stronger determinate of early growth than the food reserves of the seed. In situations where genetic differences are smaller, effects of seed weight may be relatively-larger, but it does not seem reasonable that the effect of seed weight could ever be larger enough to mask meaningful genetic differences.

Planting survival

The ability to withstand drought is critical during the first growing season after out-planting, for root surface is often drastically reduced by lifting in the nursery. The northern part of the study area is drier than the southern part during all seasons of the year so ability to withstand drought has survival value. First-year survival differences were evident in the northern planting where an

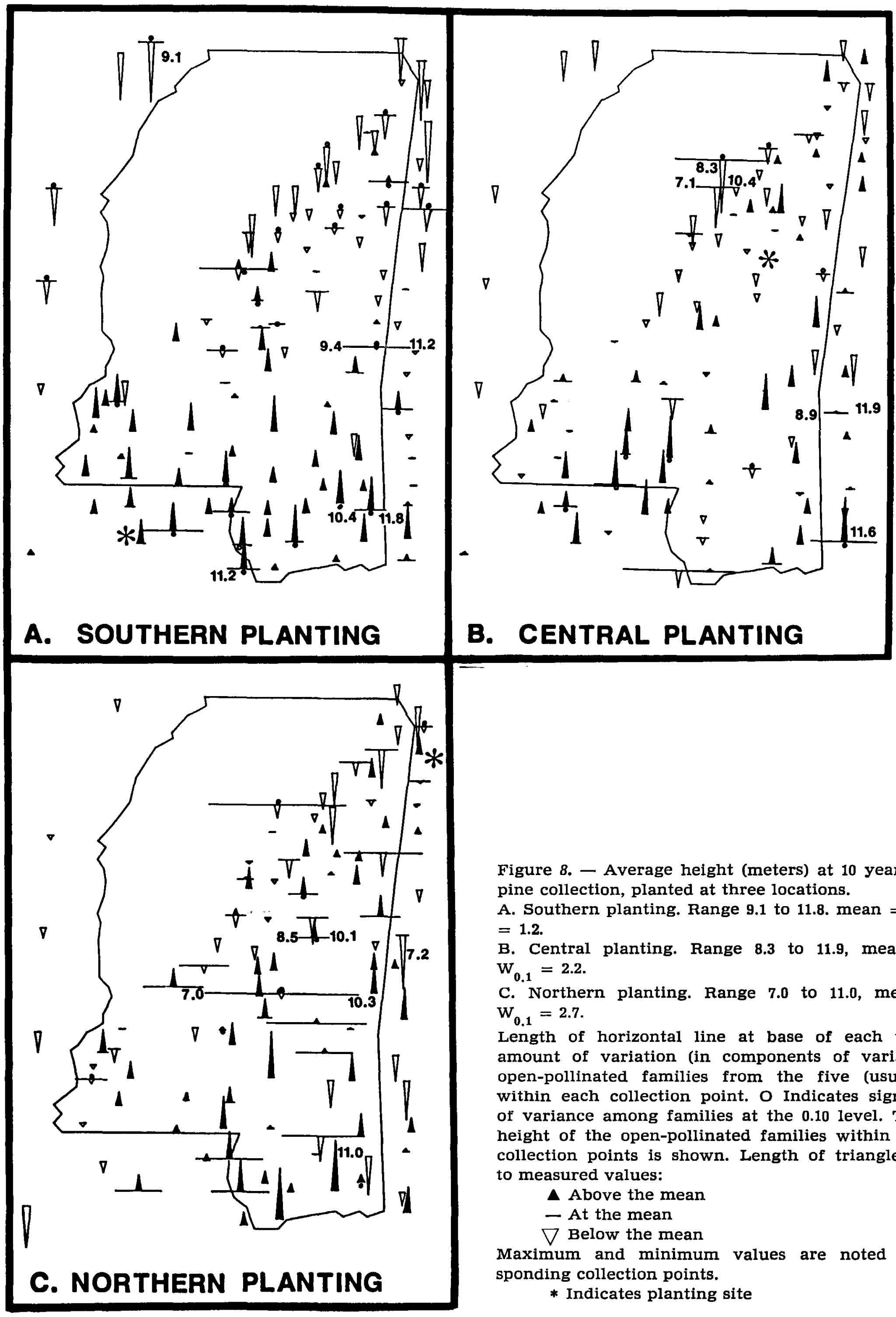


Figure 8. — Average height (meters) at 10 years for each loblolly pine collection, planted at three locations.
A. Southern planting. Range 9.1 to 11.8. mean = 10.4. Tukey's $W_{0.1}$ = 1.2.
B. Central planting. Range 8.3 to 11.9, mean = 10.1. Tukey's $W_{0.1}$ = 2.2.
C. Northern planting. Range 7.0 to 11.0, mean = 9.0 Tukey's $W_{0.1}$ = 2.7.
Length of horizontal line at base of each triangle represents amount of variation (in components of variance units) among open-pollinated families from the five (usually) parent trees within each collection point. O Indicates significant component of variance among families at the 0.10 level. The range in mean height of the open-pollinated families within two representative collection points is shown. Length of triangles are proportional to measured values:
▲ Above the mean
— At the mean
▽ Below the mean
Maximum and minimum values are noted beside the corresponding collection points.
* Indicates planting site

usually dry season provided a severe test of drought resistance. Northern populations survived better than southern ones (about 50% versus 30%) and the transition was a smooth cline (*Fig. 6b*). This trend was evident in trees from both east and west of the Mississippi River, even though the latter area was represented by only six sampling points. The effect seems a straight-forward response to selection pressure exerted by latitude-related climatic variables.

Height growth

After one growing season in the nursery, a north-south pattern of variation in height was evident, although it was not particularly distinct (Fig. 6c). More relatively fast-growing sources were found in the southern half of the study area than in the north. However, trees near the Mississippi-Alabama coast were somewhat slower growing than other southern sources.

Differences among populations in height gradually became clearer in the field plantings but the pattern of variation was not the same in each planting; genotype by environment interaction was present. Its interpretation was complicated by differences in precision among the three plantings. In the well replicated, relatively precise southern planting where the climate was much like that of the nursery, the pattern of variation in height after 5 years (*Fig. 7a*) was similar to the nursery results (*Fig. 6c*). Southern trees were generally faster-growing than northern ones except for those originating within a few miles of the coast. Western populations were somewhat slower-growing than those from the same latitude east of the Mississippi River. Further, it appeared that trees from southwestern Mississippi and the Florida Parishes of Louisiana were somewhat slower-growing than others from the southern half of the study area. An area of slow growth was also apparent along the northwestern extremity of the range within Mississippi, and a narrow north-south band of above-average growth oriented along the axis of the Pearl River occurred in central Mississippi.

By 10 years, geographic differences in the southern planting had become very distinct (*Fig. 8a*). Exept for the sources along the coastal margin, almost all sources that ranked above the mean were from the southern half of the study area. Trees from west of the Mississippi River were shorter than eastern ones from the same latitude, and the area of slow growth along the northwestern extremity of the range in Mississippi had become distinct. The narrow northsouth band of fast growth just east of and parallel to the Pearl River in central Mississippi was still evident. The trees from southwestern Mississippi and southeastern Louisiana, which initially appeared to be slower growing, had height comparable to the other southern sources at 10 years.

After 5 years, the geographic pattern of height in the two- and three-replicated central and northern plantings was indistinct (*Fig. 7b* and *c*). However, after 10 years (*Fig. 8b* and *c*), both plantings showed a strong similarity to results obtained in the southern planting (*Fig. 7a*). A notable difference in the plantings was that the southern sources were consistently taller than the average in the southern planting, but in the central and northern plantings the taller sources were from throughout the study area. It is possible that this is evidence of differences among local populations in adaption to the colder planting sites but the deficiencies in statistical precision of the central and southern plantings limit meaningful inference to only the most obvious effects.

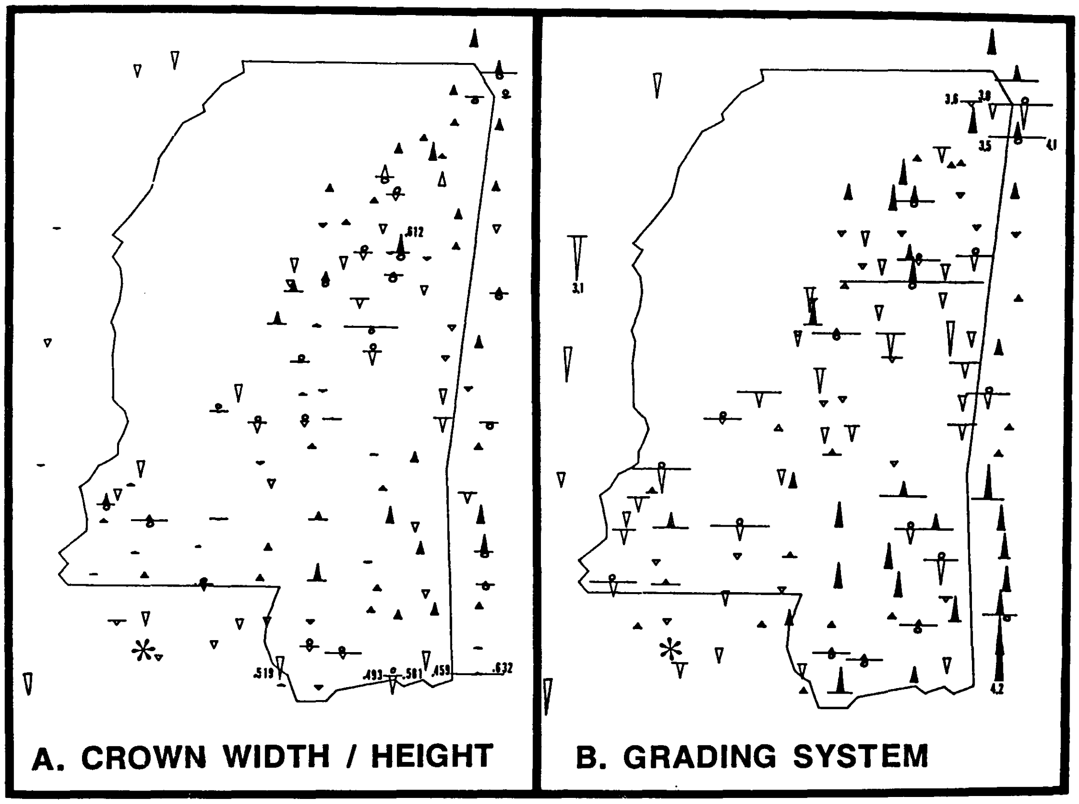
The slow growth of trees from the western collection points noted in the southern planting was also evident in the central and northern plantings, as was the slower growth of sources from the northwestern extremity of the range east of the Mississippi River.

Height variation within-stand

Geography seemed to be related to variation in height among families within collections. This relationship was best expressed in the well-replicated southern planting at 10 years where 26.6% of collection points showed significant within-stand variability (*Fig. 8a*). Sources from the southeastern part of the study area and those from a broad band extending from the geographic center of Mississippi to the northeastern corner of the state appeared to have more variability within them than did those from elsewhere in the study area.

Crown form

Both measures of tree form at 5 years (ratio of crown width to total tree height and the more subjective 1 to 5



A. CROWN WIDTH / HEIGHT

B. GRADING SYSTEM

Figure 9. — Tree form at five years for the loblolly collections at the southern location estimated by two different methods. A. Ratio of crown width to total tree height (lowest values = most columnar form). Range 0.52 to 0.61, mean = 0.57. Tukey's $W_{0.1}$ = 0.04. B. Grading system (grade 1 = most columnar form, grade 5 = bushiest form). Range, 3.1 to 4.2, mean = 3.5. Tukey's $W_{0.1}$ = 0.2. Other symbols as in *figure 8*.

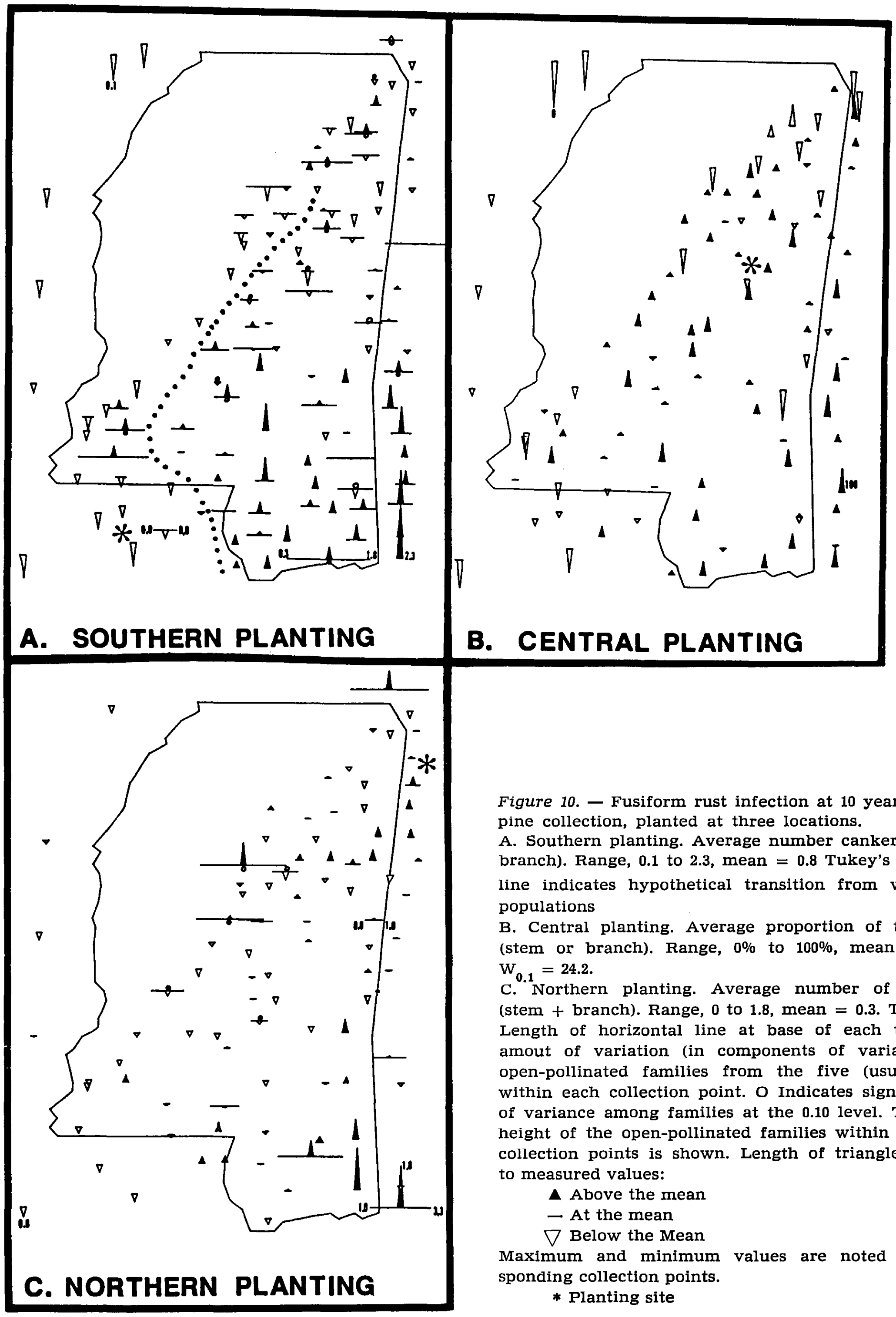


Figure 10. — Fusiform rust infection at 10 years for each loblolly pine collection, planted at three locations.
A. Southern planting. Average number cankers per tree (stem + branch). Range, 0.1 to 2.3, mean = 0.8 Tukey's $W_{0.1}$ = 0.75. Dotted line indicates hypothetical transition from western to eastern populations
B. Central planting. Average proportion of trees with cankers (stem or branch). Range, 0% to 100%, mean = 74.5%. Tukey's $W_{0.1}$ = 24.2.
C. Northern planting. Average number of cankers per tree (stem + branch). Range, 0 to 1.8, mean = 0.3. Tukey's $W_{0.1}$ = 0.98. Length of horizontal line at base of each triangle represents amout of variation (in components of variance units) among open-pollinated families from the five (usually) parent trees within each collection point. O Indicates significant components of variance among families at the 0.10 level. The range in mean height of the open-pollinated families within two representative collection points is shown. Length of triangles are proportional to measured values:

▲ Above the mean
— At the mean
▽ Below the Mean

Maximum and minimum values are noted beside the corresponding collection points.

* Planting site

visual grading method) demonstrate that western trees have less columnar crowns (higher ratio of crown width to total tree height) than those from the eastern part of the study area (*Fig. 9a, b*). However, as is the case with other traits, the dividing line between east and west is not the Mississippi River. Trees from southwestern Mississippi and southeastern Louisiana ranked with the western population with respect to crown form. The higher proportion of local populations of more columnar forms (above the planting mean) by both measures is found east of a line bisecting Mississippi from north to south.

Unlike the situation for sweetgum, where the two form traits were patterned similarly, form of the pine appears to be independent of height. The geographic variation pattern of height in pine had a north-south component that is lacking in the crown form traits.

Fusiform rust infection

After 10 years, sources from west of the Mississippi River, from the loess hills along the east side of the floodplain, and from southeastern Louisiana in the southern planting were consistently below the planting mean infection level — that is, they were relatively resistant

(*Fig. 10a*). Sources from the extreme southeastern part of the study area and along the western edge of the Tombigbee River drainage were especially susceptible to rust — i. e., local population means were consistently above the plantation mean. Trees from near the Gulf of Mexico were also consistently above the plantation mean, whereas those from farther inland were below the mean.

The pattern of rust incidence among the sources in the central planting was essentially random (*Fig. 10b*). This was undoubtedly complicated by the presence of an infection gradient in this planting (Snow et al., 1986), which contributed to error. However, in this two-replicated planting, as in the southern planting, the trees from west of the Mississippi River and southeastern Louisiana were consistently below the plantation mean for proportion of stem cankers. Also, there was a tendency for resistance along the eastern boundary of the Mississippi River floodplain, but it was not expressed as well in that planting as it was in the southern planting.

The pattern of rust resistance or susceptibility of sources in the three replicated northern planting was very similar to the pattern in the southern planting (*Fig. 9c*). Sources from a small area in northeastern Mississippi and adjacent parts of Alabama had consistently high infection in this planting, an effect not seen in the other two plantings.

The pattern of rust resistance of seed sources in this study agreed in a general way with the pattern in other wider ranging but less intensive studies (Wells and Wakely, 1966; Grigsby, 1977). In general, sources west of the river and sources from southeastern Lousiana, specifically Livingston Parish, were resistant; sources from east of this area were susceptible to fusiform rust.

This study gives a more detailed picture of the nature of the transition from resistant sources in the west to susceptible sources in the east. Previous studies have emphasized the differences between the eastern and western loblolly population created by the isolating effect of the Mississippi River valley (Wells and Wakely, 1966; Grigsby, 1977). It is obvious in this study, however, that the Mississippi River is not the dividing line between resistant sources in the west and susceptible sources in the east (*Fig 10a*). The division appears to be east of the river valley, especially in the deep south, where the border between Louisiana and Mississippi closely approximate the border between resistant and susceptible sources, respectively.

Geographic variation in rust resistance of loblolly pine is not well related to disease incidence of the four rust-resistant populations — east Texas, Arkansas, Maryland, and southeast Louisiana (Wells and Wakely, 1966) — only the southeast Louisiana source occurs in an area of high rust incidence (Squillace, 1976). In the area of highest disease incidence — i. e., central Alabama, central Georgia, and central South Carolina, the incidence of individual trees resistant to rust is rare. The notable decrease in rust incidence as one moves west of the Mississippi River can be partially explained by the increase in resistance of the native populations. The same pattern occurs in plantations of slash pine (Squillace, 1976), however, which are not native west of the river. There is no geographic variation in rust resistance in slash pine (Snyder et al., 1967); all sources are uniformly susceptible.

Although the presence of oaks (*Quereus sp.*), the alternate host for the rust fungus, is undoubtedly important, climate seems to be the limiting factor for the occurrence of the disease: cold at the northern limits of the loblolly distribution, heat at the southern limits (central Florida), and moisture at the western limits (Symposium on Management of Fusiform Rust, 1977). Very high humidity at the time of infection of the pine host is a requirement for successful transmission of the disease (Snow and Froelich, 1968). Thus, in east Texas, the high incidence of individuals resistant to the disease occurs in an area where the climate is too dry to favor occurrence of the disease.

Introgression which shortleaf pine has often been cited as a source of the resistance to fusiform rust in loblolly pine (Hare and Switzer, 1969; Florence and Hicks, 1980). In the "typical" loblolly seed sources used in the South-wide southern pine seed source study, however, Wells et al. (1979) could find no evidence for introgression of shortleaf genes in either the resistant or nonresistant populations. The isolated populations of loblolly pines west of the main loblolly range — known as the "lost pines" — are quite resistant to rust (Grigsby, 1977), even though natural shortleaf does not occur in or near these stands. Introgression could have been the ultimate source of resistance in loblolly in the distant past, but it is difficult to explain how the genes conferring resistance could become so widespread in the western populations without some kind of selection pressure in the past.

Discussion

Climatic conditions of the past can serve to explain current variation in loblolly pine and sweetgum. The anamolous pattern of rust resistance in loblolly pine, for instance, may make much more sense in the context of past geological history.

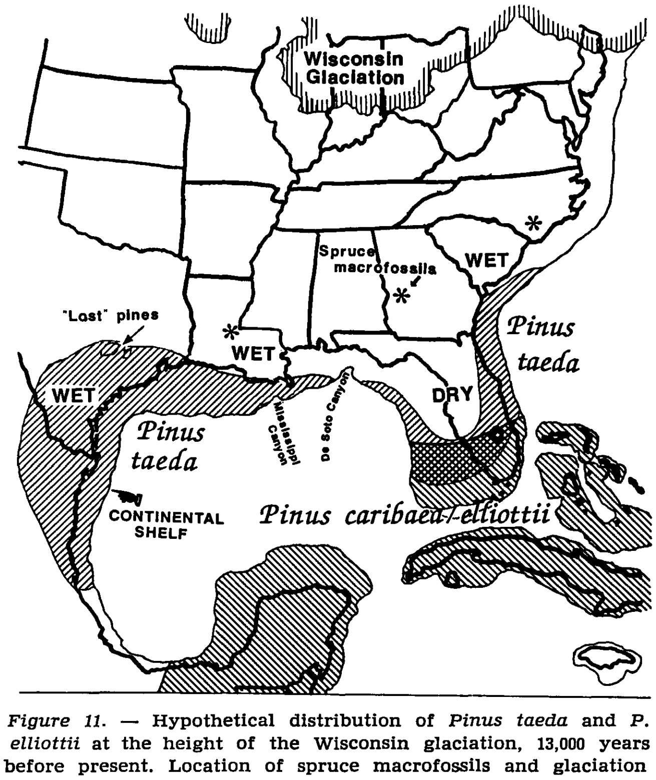


Figure 11. — Hypothetical distribution of *Pinus taeda* and *P. elliottii* at the height of the Wisconsin glaciation, 13,000 years before present. Location of spruce macrofossils and glaciation from Watts (1983). Geological features adapted from Murray (1961). *Pinus elliottii* and *P. caribaea* may have been a single species at that time, and are thus shown.

The location of loblolly pine during the height of the Wisconsin glaciation 13,000 years ago, is uncertain, as macro-fossils of southern pines from this era have not been identified. A boreal forest including spruce (*Picea* sp.) and jack pine (*Pinus banksiana* Lamb.) existed as far south as central Lousiana, central Georgia, and coastal North Carolina (*Fig. 11*), (Watts, 1983). It seems likely that the natural range of loblolly was far south of its present location. Sea level was 100 m lower than it is now, exposing the continental shelf, which was undoubtely forested.

The Mississippi River Valley may not have presented the barrier to gene flow among the eastern and western populations of loblolly pine that it does presently. The 100-m drop in the river to the ancient sea level would suggest that the river had been very fast flowing, probably in a very narrow valley — the underwater geological feature known today as the Mississippi Canyon (Murray, 1961). The alluvial plain-recognized today as the Mississippi Delta did not exist. Rather, it is more likely that a geological feature to the east of the Mississippi, the Desoto Canyon, acted to isolate eastern and western populations (*Fig. 11*).

There is ample evidence that the climate in central Florida was relatively dry during the Pleistocene (Watts, 1983). The climate to the north and west of Florida, however, was probably much wetter (Overpeck et al., 1989). In our hypothesized loblolly pine distribution during the Pleistocene (*Fig. 11*), conditions could have been optimum for selection for fusiform rust resistance in the western population (the eastern lobe of which became the resistant Livingston Parish population) and the northern Atlantic coast populations (which, after migration northward, would have become the resistant Maryland population) because of the moist conditions. There would have been no selection pressure for resistance in the Florida population, which would have become the susceptible Alabama-Georgia-South Carolina populations after northward migration.

This also explains the lack of geographic variation in rust resistance in slash pine (*P. elliottii*). It also occurs naturally in Livingston Parish, Louisiana, but not west of the Mississippi. It is probably a relatively recent newcomer to the upper Gulf Coast, having originated from a non-resistant population in south Florida. Like the loblolly from this area, it is not resistant.

Sweetgum occurs farther north than does loblolly pine (*Fig. 1*), but it is also quite likely to have occurred much farther south than currently during the Pleistocene. It is not currently sympatric with spruce and jack pine; there is no reason to suspect that it has been in the past.

There is currently no separation into eastern and western sources in sweetgum, but such a separation could have occurred in the past. Although seed weight, nursery height, and leaf retention have a well defined north-south gradient (*Fig. 3)*, which can be explained by temperature or photoperiod variation, the tenth-year height data has an east-west component with a strong gradient in eastern Mississippi (*Fig. 4*) the same is true of crown form (*Fig. 5*). The dotted line in *figure 4a* approximates the transition between the slower than average growing western sweetgum dources and the faster-than-average growing eastern sources. If you compare this to the transition between fusiform-rust resistant western loblolly sources and susceptible eastern sources (*Fig. 10a*), it is evident that the locations of two transition zones are very similar. This could be an area where two isolated populations coalesced. The gradient is difficult to explain otherwise, as the transition does not conform to any current climatic or geological feature.

Conclusions

Variation in loblolly pine expresses the effects of climate-related selection pressure in the evolution of many of the traits studied here and elsewhere (Wells and Wakeley, 1966; Sluder, 1980; Goggans et al., 1972). Such traits generally vary in a clinal, north-south pattern. Compared to populations from the northern part of the study area, southern populations have smaller seed and are more resistant to fusiform rust and drought. This variation is continuous but has a very steep gradient of change east of the Mississippi River and loblolly pine from southwestern Mississippi and southeastern Louisiana is more like the western population than the eastern. The formidable isolation barrier of the Mississippi River and its associated floodplain may not have been an important factor during the Pleistocene, and the southeastern Louisiana populations may have been part of the western population at that time, as indicated by the variation in rust resistance, form, and seed weight.

Sweetgum exhibits latitude-related variation in several traits (1-year height, time of leaf fall, and seed weight) and such variation is clinal. The seed weight pattern is the same for both sweetgum and loblolly pine, but the height variation pattern of sweetgum, unlike loblolly pine, has an east-west component in the area east of the Mississippi River. Variation in both loblolly pine and sweetgum may show the effects of an isolation into two populations during the Pleistocene, an isolation unrelated to the present location of the Mississippi River Valley.

Literature

Baker, H. G.: Seed weight in relation to environmental conditions in California. Ecology **53**: 997—1010 (1972). —— Delcourt, P. A. and Delcourt, H. R.: Vegetation maps for eastern North America: 40,000 yr B. P. to the present. In: Proc. Geobotany Conference. Plenum Press, New York. 561 p. (1981). —— Florence, L. Z. and Hicks, Jr., R. R.: Further evidence for introgression of *Pinus taeda* with *P. echinata*: Electrophoretic variability and variation in resistance to *Cronartium fusiforme*. Silvae Genet. **29**: 41—44 (1980). —— Franklin, E. C.: Inbreeding depression in metrical traits of loblolly pine (*Pinus taeda* L.) as a result of self-pollination. NC State University Sch. For. Resour. Tech. Rep. **40**. Raleigh, NC. 19. p. (1969). —— Goggans, J. F., Lynch, K. F. and Garin, G. I.: Early results of a loblolly pine seed source study in Alabama. Auburn Univ. Agric. Exp. Stn., Res. Pap. SO-89. Auburn, AL. 10 p. (1972). —— Grigsby, H. C.: A 16-year provenance test of loblolly pine in southern Arkansas. Proc. 14th Southern Forest Tree Improvement Conf. p. 261—268 (1977). —— Hare, R. C. and Switzer, G. L.: Introgression with shortleaf pine may explain rust resistance in loblolly pine. USDA Forest Serv., Res. Note SO-88, South. Forest Exp. Stn., New Orleans, LA. 2 p. (1969). —— Kraus, J. F. and Squillace, A. E.: Selfing vs. outcrossing under artifical conditions in *Pinus elliotti* Engelm. Silvae Genet. **13**: 72—76 (1964). —— McWhorter, J. C.: Climatic patterns of Mississippi. Miss. State Univ. Agric. Exp. Stn., Bull. 650. State College, MS. 24 p. (1962). —— Murray, G. E.: Geology of the Atlantic and Gulf Coastal province of North America. Harper, New York. 692 p. (1961). —— Overpeck, J. T., Peterson, L. C., Kipp, N., Imbrie, J. and Rind, D.: Climate change in the circum-North Atlantic region during the last deglaciation. Nature **338**: 553—557 (1989). —— Perry, T. O.: Maternal effects on the early performance of tree progenies. *In*: (M. G. R. Cannell and F. R. Last, eds). Tree Physiology and Yield Improvement. Academic Press, New York (1976). —— Sluder, E. R.: A study of geographic variation in loblolly pine in Georgia — 20th year results. USDA Forest Serv. Res. Paper SE-213. Southeastern Forest Exp. Stn., Asheville, NC. 26p. (1980). —— Smouse, P. E. and Saylor, L C.: Studies of the *Pinus rigida-serotina* complex. II. Natural hybridization among the *Pinus rigida-serotina* complex, *P. taeda* and *P. echinata*. Ann. Missouri Bot. Garden **60** (2): 192—203 (1973). —— Snow, G. A. and Froelich, R. C.: Daily and seasonal dispersal of basidiospores of *Cronartium fusiforme*. Phytopatology **58**: 1532—1536 (1968). —— Snow, G. A., Wells, O. O. and Switzer, G. L.: Fusiform rust gradient in a loblolly pine plantation. For. Sci. **32**: 372—376 (1986). —— Snyder, E. B., Wakely, P. C. and Wells, O. O.: Slash pine provenances tests. J. For. **65**: 414—420 (1967). —— Squillace, A. E.: Geographic patterns of fusiform rust infection in loblolly and slash pine plantations. USDA Forest Serv. Res. Note SE-232. Southeastern Forest Exp. Stn., Asheville, NC. 4p. (1976). —— Squillace, A. E. and Kraus, J. F.: Early results of a seed source study of slash pine in Georgia and Florida. Proc. 5th Southern Forest Tree Improvement Conf. 1959: 21—34 (1959). —— Strauss, S. H., Neale, D. B. and Wagner, D. B.: Genetics of the chloroplast in conifers. J. Forestry **87**: 11—17 (1989). —— Symposium on Management of Fusiform Rust: Influence of climate on fusiform rust incidence and distribution. Proc.. Symp. on Management of Fusiform Rust in Southern Pines. (Gainesville, FL., Dec. 1978). University of Florida, Gainesville, FL p. 80 (1977). —— Tukey, J. W.: Quick and dirty methods in statistics. Part II. Simple analyses for standard designs. Proc. 5th Ann. Convention, Amer. Soc. for Quality Control. pp. 189—197 (1951). —— Watts, W. A.: Vegetational history of the eastern United States 25,000 to 10,000 years ago. *In*: S. C. Porter (ed.). The Late Pleistocene. p. 294—310. Vol. I. Late-Quaternary environments of the United States. University of Minneapolis Press, Minneapolis (1983). —— Wells, O. O., Nance, W. L. and Thielges, B. A.: Variation in needle traits in provenance tests of *Pinus taeda* and *P. echinata*. Silvae Genetica **26**: 125—130 (1977). —— Wells, O. O. and Wakeley, P. C.: Geographic variation in survival, growth and fusiform-rust infection of planted loblolly pine. For Sci. Monogr. **11**, 40 p. (1966). —— Wilcox, J. R.: Sweetgum seed stratification requirements related to winter climate at seed source. For. Sci. **14** (1): 16—19 (1968). —— Wright, J. W.: Introduction to Forest Genetics. Academic Press, Inc., New York. 463 p. (1976). —— Yates, F.: The analysis of replcated experiments when the field results are incomplete. Am. J. Exp. Agric. **1**: 129—142 (1933).

A Simple and Rapid Method for Estimating Representation of Species in Spruce Seedlots using Chloroplast DNA Restriction Fragment Length Polymorphism

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Abstract

Spruce seedlots containing species mixes and hybrids of Sitka spruce (*Picea sitchensis* [Bong.] Carr.) and interior spruce (*Picea glauca* [Moench] Voss/*P. engelmanni* [Parry]) produce seedlings of unacceptable stocktype under operational nursery growing regimes in British Columbia. We have investigated the utility of chloroplast DNA (cpDNA) restriction fragment length polymorphisms for identification of the species composition of these seedlots. A *BamHI* library of Sitka spruce cpDNA was constructed in pUC8. Two clones were selected by hybridization with a 10.5kb *BamHI* fragment of white spruce cpDNA which is unique to interior spruce. One of these (pSS4) containing a 4.3kb *BamHI* fragment was tested in screening of pure and mixed seedlots of Sitka and interior spruce. The results show that this probe can be used to screen total DNA samples to reliably identify and quantify the cpDNA composition of two week old germinants using a sample size of 0.5 g and allows less than 5% species contamination to be detected. Analysis of seedlings from a hybrid seedlot showed that both chloroplast types could be found in some individuals. This result demonstrates the occurrence of hybrid individuals in seedlots and suggests that chloroplasts can be biparentally inherited in *Picea* ssp. Seedlot identification obtained with the cpDNA probe agreed with the recommended growing regimes based on the nursery performance of the seedlings.

Key words: Picea, chloroplast, DNA, polymorphism, hybrids.

Introduction

Approximately 100 million spruce seedlings are produced for reforestation operations in British Columbia annually. Seed collections from natural stands of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and interior spruce (comprising the white/Engelmann complex; *Picea glauca* [Moench] Voss/*P. engelmannii* [Parry] provide the majority of seed sown in British Columbia nurseries. The overlap of natural ranges of Sitka spruce and interior spruce and the lack of reproductive barriers to hybridization have permitted the creation of several introgression zones (Daubenmire, 1968; Roche, 1969, Krajina et al., 1982). About 6 million spruce seedlings are produced annually from seedlots collected from the coastal zone of introgression between Sitka and interior spruce. Since these seedlots are sown under the same nursery growing regime, the production of seedlings of unacceptable quality is expected due to the different cultural requirements of each species (Brix, 1972). A. reliable and cost effective means of screening these seedlots would allow mixed or hybrid seed to be grown under suitable conditions for the predominant species or to be discarded, and result in saving most of the loss now experienced. Several studies have been pursued to develop a reliable screening method which would allow accurate classification of spruce seedlots (see El-Kassaby, 1988, for review).

Recently it has been shown that chloroplast (cp)DNA restriction fragment analysis can provide a tool for determining the presence of hybrid progeny (Szmidt et al., 1987; Wagner et al., 1987; Szmidt et al., 1988; El-Kassaby et al., 1988). Analysis of inheritance in some gymnosperms have revealed that, in contrast to angiosperms, cpDNA is paternally inherited (Neale et al., 1986; Neale et al., 1989; Neale and Sederoff, 1989; Szmidt et al., 1987; Wagner et al., 1989).

We have isolated a cloned cpDNA fragment from Sitka

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