Genetic Variation in Needle Characteristics of Slash Pine and in some of its Hybrids[1])

By FRANÇOIS MERGEN

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Introduction

Following the classic experiments in transplanting by TURESSON (1922) there have been many investigations of the effects of habitat on the phenotypes of plants. At first most of these studies were mainly concerned with herbaceous plants, but now transplant experiments have become a standard technique in forestry research for the evaluation of genetic diversity in forest tree species. On the basis of such studies, the variation pattern can be described in terms of ecotypes or ecoclines, depending on whether the variation between adjacent breeding populations changes abruptly or whether the changes are gradual. Very often two trees of the same species growing at the opposite extremes of their range look alike and cannot be separated in the field on the basis of their morphological characteristics. However, by transplanting them to a new location with a different environment, these trees may react differently.

The bulk of variations within a species are the result of mutations and their recombinations, and cross pollination with other species or varieties. In our forests these phenomena are occurring continuously at a rate which depends largely on chance and environmental conditions. In forest tree improvement work a great deal of emphasis is placed on the discrete minute mutations that have resulted in qualities such as disease resistance, drought resistance, desirable form, and high oleoresin yield, to name a few.

In the forestry literature there is ample evidence of genetic variation in the forest tree species as a result of geographic location. Not only are there gross morphological differences such as height or diameter growth, but this genetic variation is also reflected in the minute differences of the individual cells of the different organs. LANGLET (1938) summarized and discussed the most significant findings with forest tree species. In almost every species investigated the various characteristics reflected to some extent the provenance of the seed source. Minute differences in biochemical properties of individual cells or organs are of importance, because these in turn are often responsible for or associated with gross morphological differences. Furthermore, the studies by WEIDMAN (1939) and STOVER (1944) added significantly to our knowledge of the effect of the habitat on the anatomical features of the leaves of conifers.

Slash pine, Pinus elliottii ENGELM., occurs naturally in the southeastern part of the United States. The southern extremes of its range are bordered by the South Florida variety, Pinus elliottii ENGELM. var. densa LITTLE and DORMAN. The range and taxonomic features of these two species were fully described in a recent publication by LITTLE and DORMAN (1954). Within its natural range, slash pine grows in mixture with loblolly pine, Pinus taeda L.; longleaf pine, Pinus palustris MILL.; pond pine, Pinus serotina MICHX.; spruce pine, Pinus glabra WALT.; and sand pine, *Pinus clausa* (CHAPM.) VASEY. Although the flowering periods of these species are staggered over a considerable interval of time, there are probably occasional opportunities for some of the sympatric species to hybridize. There are numerous reports of natural hybrids in the hard pine group, (CHAPMAN, 1922; PAPAJOANNOU, 1936; JOHNSON 1939; STOCKWELL and RIGHTER, 1946; Moss, 1945, SAITO 1951; STEBBINS 1951; WETTSTEIN 1951; ZOBEL 1951 a, 1951 b; and WRIGHT, 1956). This list will undoubtedly be extended by further investigations. It may also be pointed out that with the intensified application of silvicultural practices, and the planting of exotic species, the opportunities for interspecific cross pollinations will increase.

ANDERSON and HUBRICHT (1938) proposed use of the term "introgressive hybridization", for the backcrossing of F, hybrids to one or to both of their parents. Some of the resulting offspring are difficult to distinguish from true F, hybrids or from either of the parents. ANDERSON (1936) devised a "hybrid index," a method by which a number of characters of a putative individual hybrid or an entire hybrid swarm is compared with its typical parents. The results are scored as "a" (like species A), or as "b" (like species B), or as "i" (intermediate). In particular cases, two or more intermediate grades can be assigned. The larger the number of characters used, the more reliable will be the analysis obtained by this method. ZOBEL[2]) successfully used the ANDERSON Index to score natural hybrids between Coulter and Jeffrey pines. In addition to using the chemical properties of the oleoresin, he included such foliage characteristics as number of lines of stomata and cross-sections through stomata.

In this paper are described the results of a study of the genetic variation in needle characteristics of slash pine, Pinus elliottii var. elliottii ENGELM. The study was divided into two parts: (1) Effect of geographic origin of seed, and (2) Effect of natural interspecific hybridization with other species of pines.

For the first part such needle characteristics as number of teeth, number of resin ducts, and distribution of stomates were studied in slash pine seedlings from 12 different geographic locations. The seedling lots were grown in a uniform environment to obtain the response of the various genotypes. For the second part, the findings of the first phase were used in an attempt to obtain a diagnostic technique to verify slash pine hybrids from controlled crosses and possibly also to identify natural hybrids in the field.

Materials

The samples of pine needles were obtained from three different plantations, from natural slash pine trees growing in the vicinity of Olustee, (Baker County), Florida, and from a slash pine×loblolly pine hybrid that had been obtained through controlled pollinations.

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[1]) The author is located at the Yale University Forestry Research Center, Valhalla, N. Y. MERGEN is Assist. Prof. of Forest Genetics.

[2]) ZOBEL, BRUCE, 1951. The natural hybrid between Coulter and Jeffrey pines. Unpbl. Ph. D. dissertation, Univ. Cal. 114 pp.

I. Needles from six-month-old seedlings. The six-month-old slash pine seedlings were obtained from the "Lake City Slash Pine Seed Source Study", and were growing in a randomized block design in the Olustee Forest Experiment Station nursery in Baker County, Florida. The design of this study, as outlined in the Working Plan (MERGEN[3]), was followed very closely and the main particulars are reproduced here:

> The main objectives were (a) To test the hypothesis that distinct rates of germination are associated with different proveniences. (b) To test the hypothesis that clinal changes in growth habit in North Florida slash pine are associated with different proveniences.

Sixteen seed collections were made within the principal part of the natural range of slash pine during the fall of 1953. For each collection zone 10 cones were collected from each of 20 average dominant or codominant trees for a total of 200 cones per collection zone. The trees grew at least 200 feet from each other, to minimize the possibility of the seeds having the same parents. Excessively branchy, diseased, or insect infected trees were avoided. The seed was sown in the nursery beds during the beginning of March 1954, using a randomized block design with two replications. During September, 20 seedlings were dug from the two blocks of each source, making a total of 40 seedlings per collection zone. Twelve of the original 16 sources were collected as indicated on the map in *Figure 1*. The seedling lots were kept separate for each block and were stored in glass jars containing an FAA solution made with 75% ethyl alcohol.

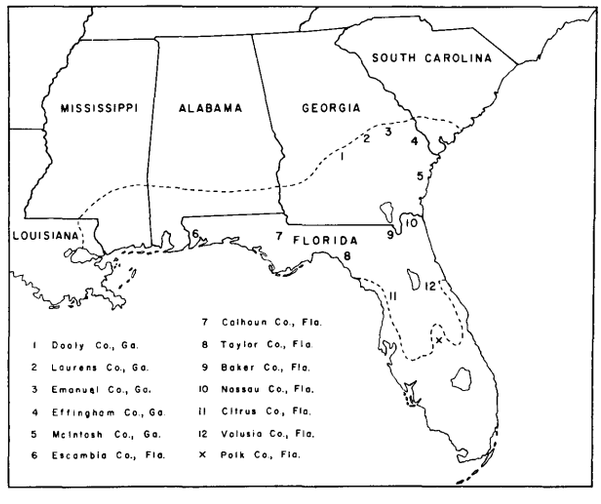

Figure 1. — Location of the 12 seed collection areas within the natural slash pine range. The seedlings were grown close to the collection point in Baker County, Florida. The natural range of slash pine is indicated by the dotted line, and the location of Polk County, Florida is marked with an X.

II. Needles from three-year-old slash pine seedlings. The needle samples from the three-year-old slash pine seedlings were collected from the Baker County, Florida, outplanting of the cooperative study of geographic sources of southern pine seed which is sponsored by the Committee on Southern Forest Tree Improvement. The details of this study are outlined in the Working Plan proposed by P. C. WAKELEY (1951). The needle samples were plucked at random from the leaders of 25 seedlings from the Baker County and Polk County sources growing in the four replications in the plantation. The needles were air-dried and stored in paper bags.

III. Needle samples from South Florida slash pine seedlings. The needle samples from two morphological types of South Florida slash pines were collected from a plantation in the Olustee Experimental Forest. This plantation contained authentic South Florida slash pine seedlings from seed collected near Homestead in Dade County, South Florida, as well as selected outstanding seedlings from a nursery bed in the Keri Nursery of the Atlantic Land and Improvement Company, near Labelle, Florida. All these seedlings were three years old at the time of collection and were selected for being outstanding in height growth; they did not exhibit the typical grass stage of South Florida slash pine. The needles were air-dried and stored in paper bags.

IV. Needles from control pollinated hybrid. The needles from a slash pine × loblolly pine hybrid resulting from controlled pollination were collected from a 10-year-old tree growing in a progeny testing plantation in the Olustee Experimental Forest. To make a comparison, needle samples were collected from five slash pine and five loblolly pine trees which grew in the vicinity of the plantation. The needles from the various trees of each species were not kept separate and they were air-dried and stored in paper bags.

V. Needles from putative natural hybrids. The needle samples from putative natural hybrids between slash and longleaf pines were collected from four-year-old trees from a natural hybrid swarm growing in an abandoned field close to Ocean Pond on the Osceola National Forest. The trees surrounding the abandoned field were slash pines with a few longleaf pines on the northern edge. An extensive reconnaissance of the surrounding forest indicated that neither loblolly nor pond pine trees were within pollination distance of the presumed mother trees. The seedlings had the general appearance of slash pines, but their pattern of height growth, and the shape of the terminal bud resembled that of longleaf pines *(Figure 2)*. Three of these seedlings were potted and transferred to a greenhouse. The needles from the putative hybrids were collected just prior to the microscopic analysis, while a composite sample of longleaf pine needles was collected from five longleaf pine trees which grew in the vicinity. The longleaf pine needles were air dried and stored in paper bags.

Methods

Resin duct counts. The number of resin ducts was counted only in the needle samples from the 12 slash pine sources of the "Lake City Geographic Seed Source Study". Freehand cross-sections were cut with a razor blade, stained with safranin, and mounted on slides. The sections were cut one centimeter from the base of mature needles to obtain a standard sample. Seventy-five needles from each of the two replications in the nursery were sampled, making a total of 150 needles from each source. The resin ducts were counted under a binocular dissecting microscope (×20), using reflected light.

Counts of Stomates. Counts of the stomates were made with a binocular microscope (×100) using reflected (obli-

[3]) MERGEN, FRANÇOIS, 1953. Slash pine *(Pinus elliottii* var. *elliotttii)* seed source study. Lake City Research Center, Mimeographed report, 13 pp.

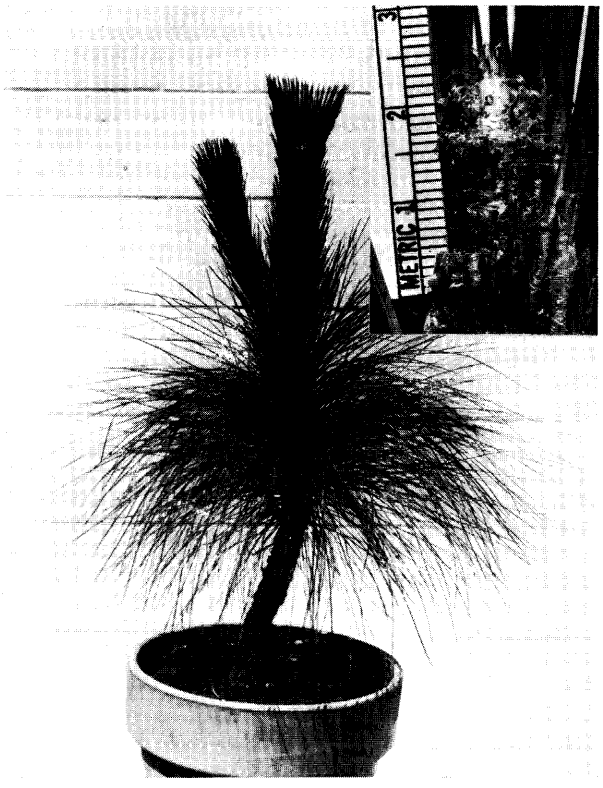

Figure 2. — Putative slash pine × longleaf pine hybrid. The insert in the upper right corner is a close-up of the terminal bud.

que) light for illumination. The needles had either two or three sides (or faces) depending on whether they grew in fascicles having two or three needles. The number of stomatal rows and the number of stomates per row were counted on needle segments, which were approximately 1 cm. long and which had been cut one inch from the base of mature needles. The counts were kept separate for the outer face (Side I) and the inner face or faces (Side II). In pines, the outer face is morphologically equivalent to the lower surface, and the inner face is the counterpart of the upper face on a leaf of a broad leaved plant. In the instances where Side II was made up of two faces, they were not kept separate but considered as one face. The length of the needle segment was measured to the nearest .01 mm. with the aid of a graduated mechanical stage. The number of stomates for each row was corrected for the difference in length between the various needle samples and was expressed as stomates per mm. for the analysis. Incomplete rows were not used to compute average values.

For the seedlings from the "Lake City Geographic Seed Source Study," fifteen needle samples from each block were used making a total of 30 needles per source. For the trees in the remainder of the study, 25 needles were measured for each sample. The needles stored in FAA were transferred to 50% ethyl alcohol before they were evaluated, those that were air-dried were soaked in water for about 12 hours to restore them to original size before they were used, whereas those from the putative natural slash × longleaf hybrid were examined immediately after collection.

Count of teeth. The teeth were counted on the edges of the segments which were used to count the stomates. They were also counted under oblique light with a binocular microscope (×100). The average number per mm. for either the two or three sides was used for the analysis.

Results

PART I: Effect of Geographic Origin of Seed

Distribution of resin ducts. The number of resin ducts ranged from one to four, with the majority of needles (84%) having 2 ducts. The seedlings from the Escambia County source were the exception with half of the needles (51%) having 3 ducts. There were only isolated instances of needles having either one or four ducts. The distribution pattern between the various sources showed that the seedlings collected from the central and northeastern counties of Florida and southeastern part of Georgia had by far the smallest number of needles with three ducts.

Distribution of Stomates. All the needles had stomates on both the inner and outer surfaces. The average number of stomates per mm. on the outer surface was 8.62 as compared to 8.87 stomates on the inner surface. Although this difference is very small and possibly of little or no biological significance, an analysis of variance of the data indicated that the difference was significant at the 0.1 per cent level. Before the data were subjected to the analysis of variance procedure they were transformed using a square root transformation to stabilize the variance. The variation between rows on the same needle was very small, and on the average the spread around the mean was less than 5 per cent.

The number of rows on Side I as compared to Side II followed a pattern opposite to that for the number of stomates per unit length. Side I had an average of 8.8 rows and the number of rows on Side II averaged 7.1. The difference was highly significant. The number of rows on needles of the same source varied considerably.

The average values for the number of stomates per mm. were plotted over latitude, longitude, average number of days without killing frost, and the average warm-season precipitation. The only distribution pattern that indicated a definite trend was that of a comparison with longitude. When the average number of stomates per mm. was plotted over longitude, a clinal variation pattern was revealed, with the sources from the eastern longitudes having a higher number of stomates than those from the western sources. This variation pattern is illustrated in *Figure 3.*

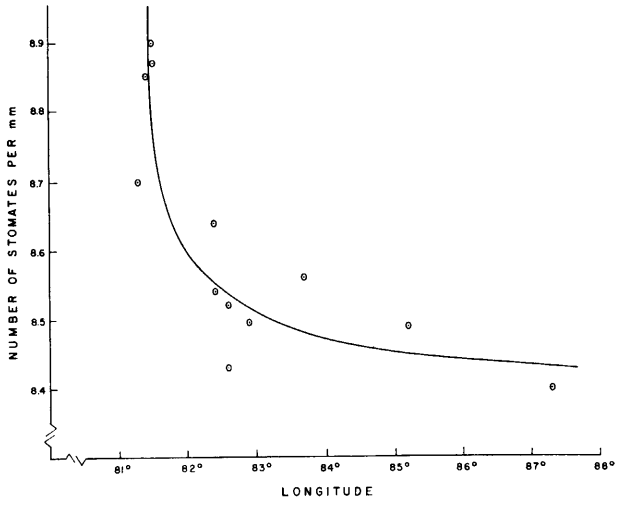

Figure 3. — Distribution of stomates in relation to longitude. A free hand curve was fitted to the data to illustrate the progressive decrease in average number of stomates in the western sources.

During the course of the study a very large number of data were gathered which made it necessary to use expedient biometrical tests. It was found that a "rankit" analysis was suitable to analyze this type of data (Bliss and Calhoun, 1954). In this type of analysis, the observations are ranked without grouping in increasing order and a rankit value is then read from a table (Fisher and Yates, 1949). The rankits are then plotted opposite their corresponding variates. A straight line is then fitted to the data. It afforded quick graphic analyses of the distribution patterns, effective tests of the normality, along with reliable estimates of the mean and standard deviation. The seedlings from the seed collected in Citrus County had the largest standard deviation based on needle means, for number of stomates per mm. of needle length on both Side I and Side II. Citrus County is located in the tension zone between North Florida and South Florida slash pine and the variation pattern was indicative of natural introgressive hybridization between these two species. For the pattern on Side II a continuous straight line could not be fitted to the data because the data on the lower (left) side of the 0 line (mean line) had a slope (standard deviation) different from that of the upper tail of the population *(Figure 4)*. This indicated a sample from a mixed population; in other words the seedlings from Citrus County are a heterogeneous population which probably resulted from introgressive hybridization with South Florida slash pines. The seedlings from Calhoun County in Florida had the smallest standard deviation when based on needle means. This possibly reflected the type of stand from which the seed was collected, namely an isolated relatively small stand which limited the possibility of outbreeding. The progressive decrease in the values for the mean from East (McIntosh County) to West (Escambia County) was also apparent with this analysis.

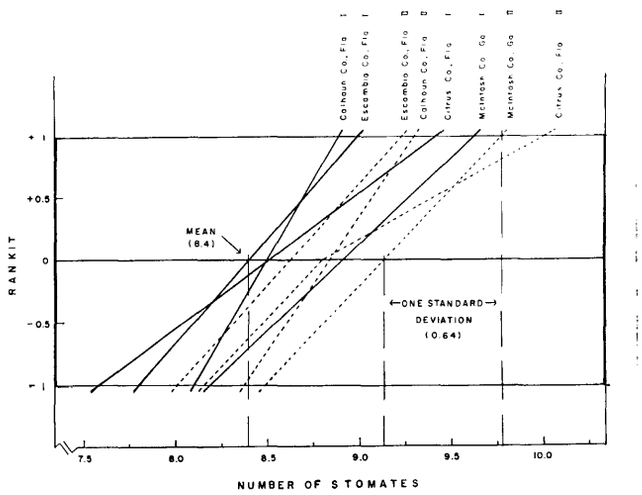

Figure 4. — Rankit analysis of the average number of stomates per mm. of needle length from four sources. Only four of the twelve sources (Side I and Side II) are reproduced here to avoid cluttering the diagram. The mean value for each source can be read directly from the intersection of the fitted line with the value for rankit O. The standard deviation is the distance between the intersection of the mean line and one rankit (+ or —) unit.

For the number of rows no distribution pattern could be established, possibly because of the great variation not only within the samples from the same source, but also between the sources. An "F" test of the variances between the sources (number of rows/number of stomates per mm.) for both Side I and Side II indicated a highly significantly greater variation in the number of rows as compared to the number of stomates per mm. The number of rows reflected the small variations in environment in the nursery beds to a greater extent than the number of stomates per unit length. This would indicate that the number of stomates per unit length is under a fairly rigid genetic control whereas the number of rows is more loosely controlled.

The average number of teeth per unit length for the various sources ranged from 2.5 to 3.1. There was no statistical difference between the means of the sources, and the variation pattern did not indicate a trend when plotted against longitude, latitude, or various climatic data. The standard deviation values based on needle means of the various sources were uniform with the exception of the Escambia County source. Seedlings from this source were much more variable in number of teeth.

PART II: Effect of Hybridization

Hybrid from controlled pollination. From the results of the evaluation of the needle characteristics in the slash pines it was felt that the characters might be suitable to verify pine hybrids from controlled pollinations and also to help diagnose putative natural hybrids. To test this hypothesis a loblolly pine×slash pine hybrid tree (F_1) from a controlled pollination was analyzed and compared with both of the parent species. The number of rows of stom-

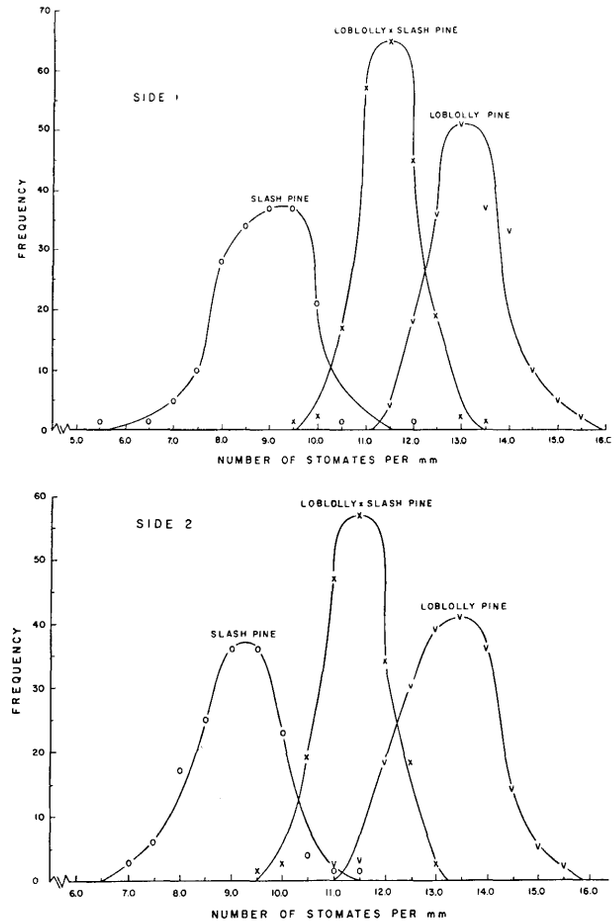

Figure 5. (A + B.) — Frequency distribution of the average number of stomates per mm. on Side I *(A)* and Side II *(B)* on a slash pine, loblolly pine, and the hybrid thereof.

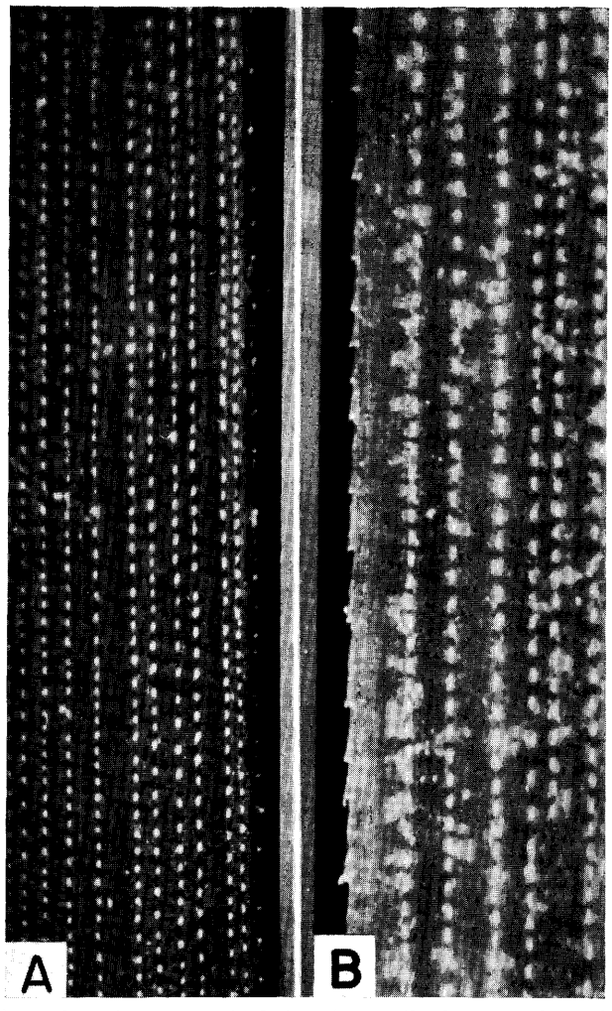

Figure 6. — Arrangement of stomates on the lower surface of a loblolly pine *(A)* needle, and a slash pine *(B)* needle.

ates, the number of stomates per unit length as well as the average number of teeth were calculated. The data on number of stomates per mm. based on individual rows of stomates are represented graphically as frequency distributions in *Figure 5 A and 5 B*. The distribution curves for slash pine and loblolly pine are based also on the number of stomates in individual rows obtained from the composite samples. Loblolly pines have a much greater number of stomates on their needles than slash pine *(Fig. 6)*. The intermediacy of the hybrid was well defined for both Sides I and II. The means in the distribution patterns of slash pine and loblolly pine were separated enough and there was only limited over-lapping in the distribution of the two species. The standard deviation

Table I. — Values for the mean, standard error of the mean, and number of observations for the stomates on both Side I and Side II on needles from loblolly pine, slash pine, and loblolly pine × slash pine.

Species	Side I			Side II		
	Mean	Standard Error of the Mean	Number of Observations	Mean	Standard Error of the Mean	Number of Observations
Loblolly pine	13.20	.0572	196	13.26	.0617	190
Loblolly pine × slash pine	11.48	.0424	209	11.45	.0476	182
Slash pine	8.82	.0867	176	9.04	.0660	152

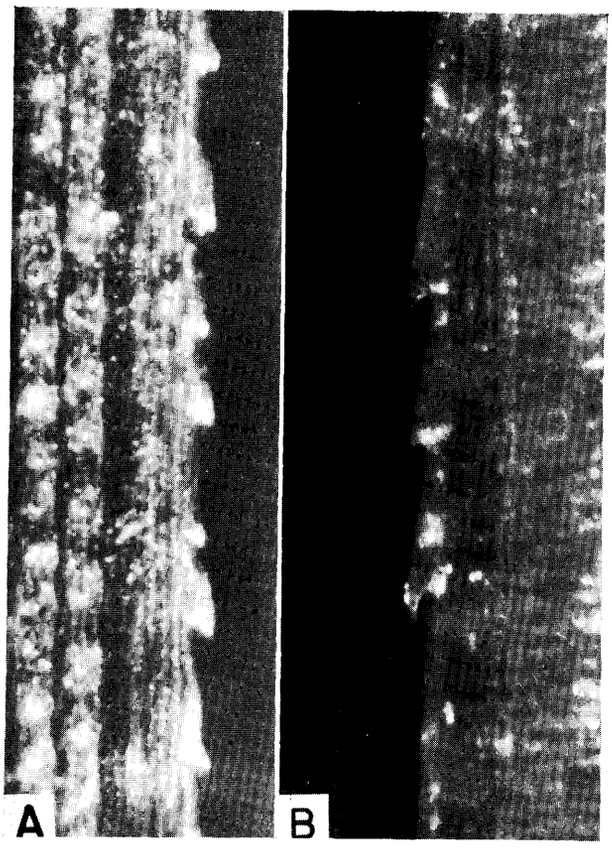

Figure 7. — Distribution of teeth along the edge of a loblolly pine *(A)* needle and a slash pine *(B)* needle.

for the hybrid based on the individual rows of stomates was also smaller than that for either of the two parental species. In *Table I* are given the values for the mean, standard error of the mean, and the number of observations for slash pine, loblolly pine, and loblolly pine × slash pine. The number of rows of stomates on the hybrid was also intermediate between the parents, The relationship was present for both Sides I and II.

Needles from the loblolly pine tree had a much greater number of teeth per unit length than those from the slash pine trees *(Fig. 7)*. Those on the loblolly needles, however, exhibited a much greater variation. The number of teeth on the needles from the hybrid were intermediate between both parent species, and the distribution pattern

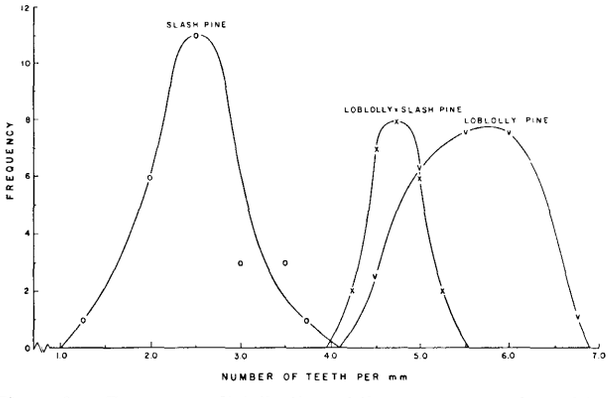

Figure 8. — Frequency distribution of the average number of teeth on slash pine, loblolly pine, and a loblolly pine × slash pine hybrid.

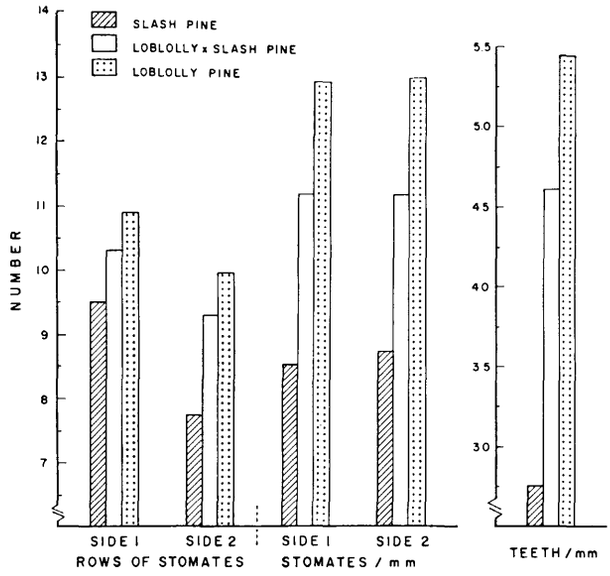

Figure 9. — Bar chart of the average values for the rows of stomates, the number of stomates, and the number of teeth for slash pine, loblolly pine, and the loblolly pine × slash pine hybrid. The intermediacy of the hybrid is clearly illustrated.

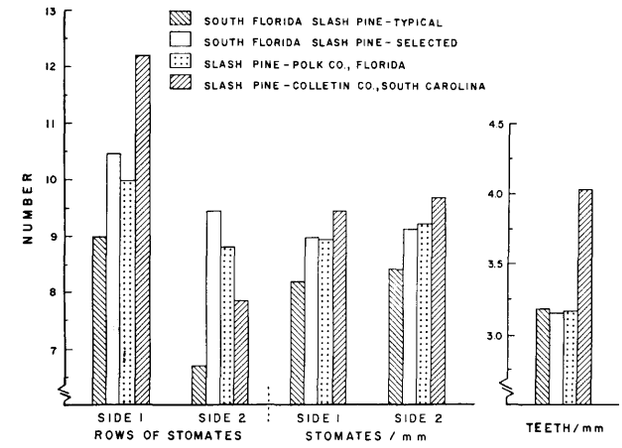

Figure 11. — Bar chart of the average values for the rows of stomates, the number of stomates, and the number of teeth for North Florida slash pine, South Florida slash pine and the two putative hybrid populations.

was similar to that for slash pine as can be seen from *Figure 8*. The number of teeth along each edge, rather than the average for each needle, was used to compile the frequency distribution. In *Figure 9* the results from the various analyses of the hybrid are combined to present an over-all picture of the intermediacy of the needle characteristics.

Putative natural hybrids. North Florida slash pine×South Florida slash pine. The difference between the means in the distribution pattern for the number of stomates per mm. in South Florida slash pines and North Florida slash pines from Baker County was relatively small *(Fig. 10 A + B)*. However, this difference in distribution was sufficient to illustrate an intermediacy in the seedlings from Polk County, and in the selected South Florida slash pines. The seedlings from Polk County were of particular interest. Their distribution based on individual measurements on a needle had a large standard deviation, and as a result both tails of the distribution extended to the extremities of both North and South Florida slash pine. The number of rows of stomates was more variable, and on Side II it was greater than that of either of the putative parent species. For Side I both of the putative hybrid sources were intermediate between their parents. The distribution pattern of the teeth on the hybrids was similar to that of the typical South Florida slash pine as can be seen from *Figure 11*. The intermediacy in the number of stomates per mm. along with their intermediate grass stage features indicate that these seedlings are hybrids between North Florida and South Florida slash pines. It was reported previously (MERGEN, 1954) that the Polk County seedlings probably are hybrids on the basis of their height growth, stem characteristics, over-all needle growth, and outward signs of frost hardening.

Slash pine×longleaf pine. The means of the distribution patterns for the number of stomates per mm. and per row of stomates in the putative natural slash pine×longleaf pine hybrids were intermediate between those of slash and longleaf pine, but with a greater standard deviation than either *(Fig. 12)*. The values from the composite slash pine sample which was used for the analysis of the loblolly pine × slash pine hybrid were used. The frequency distribution transgressed outside the upper values for longleaf pine. The average number of rows of stomates and

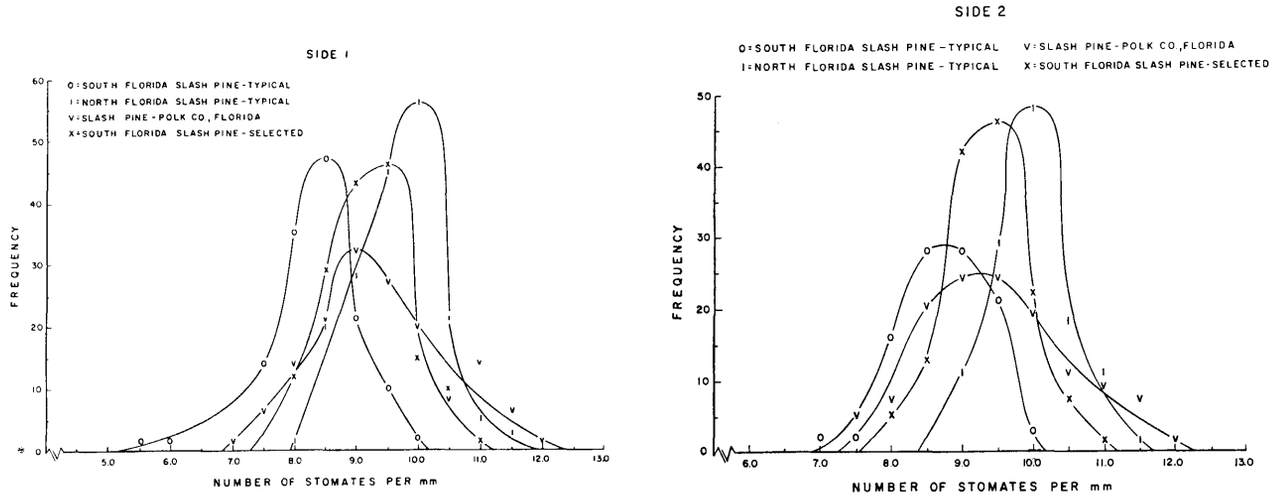

Figure 10. (A and B.) — Frequency distribution of the average number of stomates per mm on Side I *(A)* and Side II *(B)* on North Florida slash pine, South Florida slash pine, and on the two putative hybrid populations.

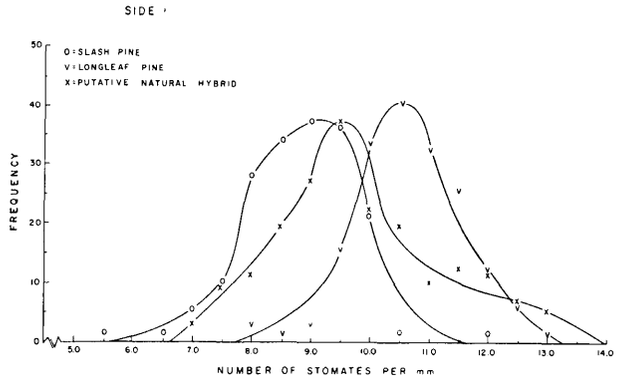

Figure 12. — Frequency distribution of the average number of stomates per mm. on slash pine, longleaf pine, and their putative hybrid.

the average number of teeth for the two putative parent species was very similar and therefore could not be used for the analysis.

Discussion

The geographic variation pattern that was observed in the number of stomates per unit length was well defined. It can be ascribed to genetic differences between the sources, because the plants were growing under a uniform environment. This variation was probably the result of mutations that occurred at some point in the population and are moving gradually through the range of slash pine. This change has probably little adaptive value, because the isobars for temperature and rainfall are running roughly in an east to west direction in this geographic location. This variation was not the effect of interspecific hybridization because the pattern was not accompanied by similar changes in number of resin ducts, or number of teeth, as would be the case if it had resulted from hybridization with another species of pine. Besides this geographic variation, it appeared that the Escambia County and the Calhoun County, Florida sources represented distinct breeding populations. The seedlings from the Escambia source had the lowest number of stomates per unit length, the largest number of needles with three resin ducts, and the needles were much more variable in number of teeth. The significantly smaller standard deviation encountered in the seedlings from the Calhoun source was probably the result of partial isolation which limited cross-pollination between trees of adjacent stands. A mild effect of inbreeding was noted before in the germination behavior of the seed from this lot which had the lowest germination percentage (Mergen and Hoekstra, 1954).

From the statistical evaluation of the variation within needles of the same plant, and between needles of different plants from the same source, and from different sources, it appears that the number of stomates per unit length is relatively independent of environmental effects. The distribution of the number of stomates in the phenotype is a very close expression of the genotype. Leaf characteristics, such as type of serration of the leaf margins or type of pubescence in *Potentilla* were studied by Clausen et al. (1940), and they noted that these could be modified only slightly or not at all by changes in the environment.

To study the genetic variation in trees and their inheritance, it is helpful if the characters can be expressed in objective terms for analysis. What often appears to be a single difference between two trees or two species, such as whitish appearance of a needle, or width of a needle, can be broken down into its primary components for the analysis. This should make it possible to obtain characters that are measurable and thus eliminate some of the personal bias in the scoring. Further, objective numerical data allow a wider use of biometrical techniques for the evaluation.

If one is dealing with features that are rigidly controlled by the genes, these characteristics can be used to study natural hybridization and introgression between species. Likewise, if these traits are stabilized in the seedlings, they are of value in studying the variation at an early age. The needle characteristics described were found to be a helpful, diagnostic tool to verify a hybrid from controlled pollination, and also to develop a "Hybridity Index" for putative natural hybrids in the field. Rollins (1944) was able to use the microscopic appearance of specialized cells of the leaf trichomes to identify natural hybrids between *Guayule* and *Mariola*. Along with other traits, the needle characters mentioned should be of value in recognizing hybrids. As with all the methods used to identify natural hybrids in the field, the evidence gathered is circumstantial.

In the analysis of the frequency distribution it became evident that the characters under study depended on multiple, rather than single, genes which showed dominance. They were intermediate between the two parent species; but in some of the putative natural hybrids this intermediacy was somewhat obscured by introgressive hybridization.

This study did not consider hybridization between slash pine and other sympatric species. The putative hybrid crosses described have already been successfully made under controlled conditions. In some parts of their natural range the flowering periods of the parent species are not separated enough to prevent their cross-pollination. During the past five years the flowering seasons of the various species have overlapped so there was adequate opportunity for cross-pollination. In addition, forestry practices, such as fire protection and planting, have enabled these species to grow and develop under conditions conducive to their cross-pollination. Through this interference with nature, some of the isolating mechanisms, which in the past have promoted the divergence of the various species, have been lessened or even eliminated.

The applicability of using the number of stomates per unit length as a diagnostic test for other species or groups of pines is being tested by comparing hybrids from controlled pollinations growing in the Arboretum at Valhalla, New York. The results of the findings will be reported later.

Acknowledgements

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Summary

The results of a study of the genetic variation in needle characteristics (number of teeth, number of resin ducts, and distribution of stomates) of slash pine, *Pinus elliottii* var. *elliottii* Engelm. are described. The study consisted of two parts: (1) Effect of geographic origin of seed, and (2)

effect of natural interspecific hybridization with other species of pine.

1. The number of resin ducts in the needles of seedlings collected from 12 geographic locations ranged from one to four, with the majority having 3 ducts. The seedlings collected from the central and northeastern counties in Florida and the southeastern part of Georgia had the smallest number of needles with three ducts.

2. The average number of stomates per mm. on the outer surfaces was slightly less than that on the inner surfaces (8. 62 versus 8. 87).

3. There was a geographic variation pattern in the seedlings from the 12 geographic locations when the average number of stomates per mm. was plotted over longitude. The seedlings from the eastern longitudes had a higher number of stomates per mm than those from the western sources.

4. The distribution in the average number of stomates per mm. in the needles from Citrus County, Florida was indicative of natural introgressive hybridization with South Florida slash pine, *Pinus elliottii* var. *densa* LITTLE and DORMAN.

5. The average number of teeth per unit length for the various sources ranged from 2.5 to 3.1. The variation patterns did not indicate a trend when plotted against longitude, latitude, or various climatic data.

6. From the statistical evaluation of a large number of samples , it appeared that the number of stomates per unit length is relatively independent of environmental effects and that it is under fairly rigid genetic control.

7. When compared to its parents, a loblolly pine × slash pine hybrid had intermediate values in the needle characteristics studied.

8. It was proposed to apply numerical values to qualitative traits of putative hybrids so that biometrical tests can be applied to study their degree of hybridity.

9. The method described was found suitable to evaluate putative natural hybrids between slash pine × longleaf pine, and slash pine × South Florida slash pine.

Zusammenfassung

Titel der Arbeit: *Genetische Variabilität von Nadeleigenschaften der Pinus elliottii und einiger ihrer Bastarde.* —

Es werden Untersuchungsergebnisse über die genetische Variabilität von Nadeleigenschaften (Anzahl der Zähne und der Harzkanäle, Anordnung der Spaltöffnungen) von *Pinus elliottii* var. *elliottii* ENGELM. beschrieben. Die Untersuchungen gliedern sich in zwei Teile: 1. Einfluß der Herkunft des Saatgutes, 2. Einfluß der natürlichen interspezifischen Kreuzung mit anderen Kiefernarten.

1. Bei Sämlingen von 12 verschiedenen Herkünften variierte die Anzahl der Harzkanäle in den Nadeln von 1—4. Am häufigsten wurden 3 Kanäle festgestellt. Die Sämlinge aus dem zentralen sowie aus dem nordöstlichen Teil Floridas und die aus dem südöstlichen Teil Georgias wiesen am wenigsten Nadeln mit 3 Harzgängen auf.

2. Der Mittelwert der Spaltöffnungszahlen pro mm war auf der Außenseite der Nadeln niedriger als auf der Innenseite (8,62 gegenüber 8,87).

3. Je nach der geographischen Länge der Herkunft waren bei Sämlingen der 12 untersuchten Herkünfte Unterschiede in der Anzahl der Spaltöffnungen nachzuweisen. Die Sämlinge der östlicher gelegenen Herkünfte hatten eine höhere Anzahl Spaltöffnungen als die westlicheren Herkünfte.

4. Die Verteilung der Mittelwerte der Spaltöffnungen pro mm bei der Herkunft Citrus County/Florida war ein Hinweis auf natürliche introgressive Bastardierung mit *Pinus elliottii* var. *densa* LITTLE and DORMAN.

5. Der Mittelwert der Zähne pro Längeneinheit variierte bei den verschiedenen Herkünften zwischen 2,5 und 3,1. Es konnte aber keine Abhängigkeit dieser Werte von Längen- oder Breitengraden oder verschiedenem Klima festgestellt werden.

6. Nach der statistischen Auswertung einer großen Anzahl Proben ist anzunehmen, daß die Anzahl Spaltöffnungen pro Längeneinheit von Umwelteinflüssen relativ unabhängig ist und einer strengen genetischen Kontrolle unterliegt.

7. Die Nadeleigenschaften des Bastards *Pinus palustris* MILL. × *P. elliottii* var. *elliottii* ENGELM. sind intermediär gegenüber den Elternarten.

8. Es wird vorgeschlagen, qualitative Merkmale vermeintlicher Bastarde zahlenmäßig zu fassen, um biometrische Untersuchungen zur Feststellung des Bastardgrades anstellen zu können.

9. Die beschriebene Methode erwies sich als geeignet zur Beurteilung mutmaßlicher natürlicher Bastarde zwischen *Pinus elliottii* var. *elliottii* ENGELM. und *Pinus palustris* sowie zwischen *Pinus elliottii* var. *elliottii* ENGELM. und *Pinus elliottii* var. *densa* LITTLE and DORMAN.

Résumé

Titre de l'article: *Variations héréditaires dans les caractères des aiguilles chez le «Slash pine» et quelques uns de ses hybrides.*

L'étude porte sur la variation héréditaire des caractères des aiguilles (nombre de dents, nombre de canaux résinifères, répartition des stomates) du «Slash pine», *Pinus elliottii* var. *elliottii* ENGELM. Elle comprend deux parties: (1) influence de l'origine géographique des graines et (2) influence d'hybridations interspécifiques naturelles avec d'autres pins.

1. Le nombre des canaux résinifères dans les aiguilles de semis récoltés dans 12 stations différentes varie de 1 à 4, avec un maximum pour 3 canaux. Les semis provenant des comtés du centre et du Nord de la Floride et du Sud de la Géorgie ont le plus petit nombre d'aiguilles à 3 canaux.

2. Le nombre moyen des stomates par mm sur la face externe est un peu plus faible que sur la face interne (8, 62 contre 8, 87).

3. On a constaté l'existence d'une relation entre le nombre moyen des stomates et la longitude de la station d'origine: les semis des provenances les plus à l'Est ont plus de stomates par mm que ceux des provenances situées le plus à l'Ouest.

4. La distribution des nombres moyens de stomates par mm dans les aiguilles des semis de Citrus County, Florida, indique l'existence d'une hybridation introgressive naturelle avec le «Slash pine» du Sud de la Floride, *Pinus elliottii* var. *densa* LITTLE et DORMAN.

5. Le nombre moyen de dents par unité de longueur varie, suivant les provenances, de 2,5 à 3,1. Il n'existe pas de relation de cette grandeur avec la longitude, ni avec une donnée climatique quelconque.

6. On peut conclure, de l'évaluation statistique d'un grand nombre, d'échantillons, que le nombre de stomates par unité de longueur est relativement indépendant de l'action du milieu et dépend assez étroitement de facteurs génétiques.

7. Les caractéristiques des aiguilles d'un hybride «Loblolly pine» *(Pinus taeda* L.) × «Slash pine» sont intermédiaires entre celles des parents.

8. On propose de donner des valeurs numériques aux caractères qualitatifs des hybrides supposés, afin de déterminer leur degré d'hybridité par des tests biométriques.

9. La méthode décrite s'est révélée efficace pour évaleur les hybrides naturels supposés de «Slash pine» avec «Longleaf pine» *(P. palustris)* et «Slash pine» avec «Slash pine» variété du Sud de la Floride.

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Die statistische Auswertung von vergleichenden Versuchen

Von Lars Strand, Vollebekk

Forstliche Versuchsanstalt Norwegens

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Die Ergebnisse eines jeden Versuches bilden die Grundlage bestimmter, im Anschluß an den Versuch zu treffender Maßnahmen. Darum ist es wichtig, die Versuche so auszuführen, daß diese Maßnahmen sinnvoll sind. Zu diesem Zwecke mag die Statistik ein wertvolles Hilfsmittel sein.

Es wird oft gesagt, die erste Voraussetzung für die Anwendung statistischer Methoden sei ein Versuchsmaterial mit vielen Beobachtungen. In Wirklichkeit liegen die Dinge umgekehrt und vielleicht sind die statistischen Methoden eben dort von größerem Nutzen, wo man nur wenige Beobachtungen zur Verfügung hat. Man wird in diesen Fällen vor falschen Folgerungen bewahrt.

Daneben hat die Anwendung statistischer Methoden einen weiteren Vorteil: Es hat sich oft gezeigt, daß Fehler der Anlagepläne aufgedeckt werden, wenn die statistische Analyse einsetzt. Dies Moment ist vielleicht ebenso wichtig, wie das erstgenannte. Ein Versuch, welcher schlecht geplant ist, kann niemals so viele Informationen liefern, wie ein gut geplanter Versuch. Betreffs der Planung von Versuchen gibt es eine reichhaltige Literatur (z. B. Fisher 1953, Cochran and Cox 1950), wo man die notwendigen Anweisungen finden kann. Die Prinzipien der verschiedenen Anlagepläne werden daher hier nicht behandelt, es soll aber ein konkretes Beispiel dazu dienen, die Notwendigkeit einer guten Versuchsplanung zu unterstreichen. Zu diesem Zwecke wird ein Versuch von Münch (1949) benutzt.

Als Münch seine Versuche anlegte, war die Kenntnis statistischer Methoden nur gering. Für die folgende Diskussion ist es notwendig, darauf hinzuweisen. Der Versuch wird benutzt, um ein Beispiel zu haben und *nicht* um Münch zu kritisieren.

Im Jahre 1932 wurde im Forstamt Spechtshausen, Abt. 141, ein Versuch mit Absaaten verschiedener Mutterbäume ausgelegt. Insgesamt 16 Sorten gingen in den Versuch ein, der außerdem Kontrollparzellen mit Pflanzen einer bestimmten Herkunft enthielt. Die Teilflächen der Sorten umfaßten 6 Reihen, die Kontrollteilflächen 3 Reihen. Jeder Sorten-Teilfläche war eine Kontrollteilfläche benachbart. Die letzte Aufnahme des Versuches wurde ausgeführt, als die Pflanzen 14 Jahre alt waren.

Ziel des Versuches war es zu untersuchen, ob die verschiedenen, in den Versuch eingegangenen Populationen identisch sind. Darum interessieren uns die beobachteten Höhen nur in dem Maße, als sie Aussagen über die „wah-