— LUNDKVIST, K.: Inheritance of acid phosphatase isozymes in Picea abies. Hereditas 79: 221–226 (1975). — MUHS, H. J.: Distinction of Douglas-fir provenances using peroxidase-isoenzyme-patterns of needles. Silvae Genetica 23 (1-3): 71–77 (1974). — NEI, M.: Genetic distance between populations. Amer. Natur. 106 (949): 283–292 (1972). — PRAKASH, S., LEWONTIN, R. C. and HUBBY, J. L.: A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal, and isolated populations of Drosophila pseudoobscura. Genetics 61: 841–858 (1969). — RASMUSON, B. and RUDIN, D.: Variations in esterase zymogram patterns in needles of Pinus silvestris from provenances in northern Sweden. Silvae Genetica 20 (1-2): 39—41 (1971). — RUDIN, D., ERIKSSON, G., EKBERG, I. and RASMUSON, M.: Studies of allele frequencies and inbreeding in Scots pine populations by the aid of the isozyme technique. Salvae Genetica 23 (1-3): 10–13 (1974). — RUDIN, D. and RASMUSON, B.: Genetic variation in esterases from needles of Pinus silvestris L. Hereditas 73: 89–98 (1973). — SELANDER, R. K., HUNT, W. G. and YANG, S. Y.: Protein polymorphism and genic heterozygosity in two European subspecies of the house mouse. Evolution 23: 379–390 (1969). — SHAW, C. R.: Electrophoretic variation in enzymes. Science 149: 936–943 (1965). — SILEN, R. R.: Pollen dispersal consideration for Douglas-fir. J. For. 60 (11): 790–795 (1962). — SNEDECOH, G. W. and COCHRAN, W. G.: Statistical methods. 6th ed., Iowa State University Press, Ames. 593 p. (1967). — STERN, K.: Die Bedeutung Genetischer Polymorphismen für Forstgenetik und Forstpflanzenzüchtung. Allg. Forst. u. J.-Ztg. 139 (9): 199–204 (English translation by Foreign Languages Division, Dept. of the Secretary of State, Translation Bureau, Canada, 1971). (1968). — TIGERSTEDT, P. M. A.: Studies on isozyme variation in marginal and central populations of Picea abies. Hereditas 75: 47-60 (1973). — WAGNER, R. P. and SELANDER, R. K.: Isozymes in insects and their significance. Ann. Rev. Entomol. 19: 117–138 (1974).

# Genotype x Environment Interaction and Genotpic Stability in loblolly pine

## I. General introduction and description of the experiment[*]

By F. OWINO[1] and B. ZOBEL[2]

(Received July / September 1976)

### General introduction and description of the experiment

Genotype X environment interactions — the differential response of genotypes to varied environments, when present, introduce biases in genetic and environmental improvement predictions. There are two ways by which a breeder can circumvent the undesirable consequences of such biases: (1) by grouping the genotypes according to their regions of optimal adaptation and (2) by changing the testing design to minimize such interaction effects through manipulation of plot size, plot shape, number of replications and number of test environments. Tree breeders favor selection and breeding for wide adaptation partly because of the great variation in site classes for which they breed and partly to avoid working with narrowly adapted populations with restricted genetic bases that might prove vulnerable to pests and adverse future environments. Also, there are practical limitations as to how far testing designs can be manipulated to reduce the impact of genotype X environment interaction in tree breeding; the long duration of the tests and the high hazards of the forest environment often determine the lower limit of the number of replications and the number of trees per plot. Furthermore, difficulties of obtaining seed of all the families for testing at any one time is a well known constraint on the number of test evironments possible.

While selecting and testing genotypes for broad adaptability, it is important to obtain estimates of the amount of bias in genetic gain prediction due to genotype X environment interaction effects. Of particular importance in the widely adopted population improvement breeding strategies is the ratio of additive genetic variance X environment interaction to additive genetic variance. To date, tree breeding experiments reported in the literature have not been big enough and well designed to provide the type of information referred to here because of (a) insufficient specification of genotypes studied, (b) insufficient numbers of genotypes studied or (c) insufficient numbers of test environments studied.

The experiment reported here was designed to provide information on the relative size and importance of genotype X environment interaction and genotypic stability in a genetic improvement program with loblolly pine. The experiment had its origin in the controlled crosses made by WOESSNER in 1964 and 1965 to explore the possibilities of heterotic combinations from wide crosses. The original experimental objective of value assessment for wide crosses was included in the reported investigation for completeness. The investigation was designed to answer the following questions:

1. Do open-pollinated progeny, local single crosses, and wide crosses differ in genotypic stability?
2. What is the most suitable genotypic stability parameter for use in loblolly pine family comparisons?
3. Do wide crosses show heterosis and, if so, how consistent is the heterosis over several environments?
4. To what extent can the genotype X environments interaction observed reduce the repeatability of family means over environments?
5. To what extent can genotype X environment interaction bias estimates of components of genetic variance and consequently genetic gain predictions?
6. Is there evidence of genetic differentiation within the loblolly pine range which could be utilized to broaden the gene base of breeding populations?

---

[*] This work was carried out in partial fulfilment of the requirements for the Ph. D. degree at North Carolina State University, 1975.

[1] Lecturer, Botany Department, University of Nairobi, Kenya.

[2] Professor of Forest Genetics, North Carolina State University, Raleigh, U.S.A.

## Materials and Methods

### *Mating design*

The crosses on which these studies are based, were made by Woessner in 1964 and 1965 to explore the possibilities of heterotic combinations from wide crosses. A thorough description of the mating pattern has been given by Woessner (1968, 1972).

Four seed orchard trees indigenous to the North Carolina Piedmont (Hoerner-Waldorf seed orchard) and four seed orchard trees indigenous to the North Carolina Coastal Plain (Weyerhaeuser seed orchard) were crossed with pollen of seed orchard trees from ten widely separated geographic areas according to design II of Comstock and Robinson (1948). *Figure 1* shows the area of origin of the parents used while *Table 1* shows the successful sets of crosses. A half dialled design was used for the within orchard crosses. Also, open-pollinated seed of 32 pollen and seed parents were collected and included in the study.

*Table 1.* — Sets of inter- and intra-population control-pollinated crosses.

| Male parents and State | Female parents | |
|---|---|---|
| | Hoerner-Waldorf seed orchard | Weyerhaeuser seed orchard |
| Bowaters (Tenn.) | | × |
| Champion (S.C.) | | × |
| Continental Can (Ga.) | × | × |
| Continental Can (La.) | × | × |
| Weyerhaeuser (N.C.) | × | × |
| Union Camp (Ga.) | × | |
| Westvaco (N.C. -wet site) | × | |
| Westvaco (S.C.) | × | |
| Texas | × | × |
| Hoerner-Waldorf (N.C.) | × | × |
| Rimberly-Clark (Ala.) | × | |

### *Field design*

The seedlings were outplanted at 12 locations in five Southeastern states. *Figure 2* shows these test locations and *Table 2* gives a summary by County and State, physical division, soil type, site preparation, and number of replications at each of the nine locations used in the present analyses. The other three locations were excluded from the analyses because of the extreme variability in growth due to observable causes such as accidental fire (Georgetown, S.C.), severe competition with indigenous hardwoods (Durham, N.C.), and extremely poor drainage (Beaufort, N.C.).

Outplanting was done in late February and early March, 1968, at all locations except in Tyrrell County where this was done in late May 1968. Randomized complete block design with 10-tree row plots planted at 9' × 9' spacing was used at all locations assessed except for the planting in Durham County which have 2-tree row plots.

## Data Collection

Field assessments were done at the end of the fourth growing season in the Spring of 1973. For each tree, five traits were recorded as follows:

1. Tree status — on a code that specifies wether a tree is dead, missing, not planted, or a filler.
2. Total height — to the nearest one-half foot of the leading shoot.
3. Fusiform rust infection score — on a code that ranges from 1 (no infection) to 5 (the most severe infection).
4. Crown from score — on a code that ranges from 1 (a good, uniform, dense crown with thin, horizontal branches) to 6 (forked, heavily branched, and sparse crown).
5. Stem straightness score — on a code ranging from 1 (no or very slight crook) to 6 (very crooked).

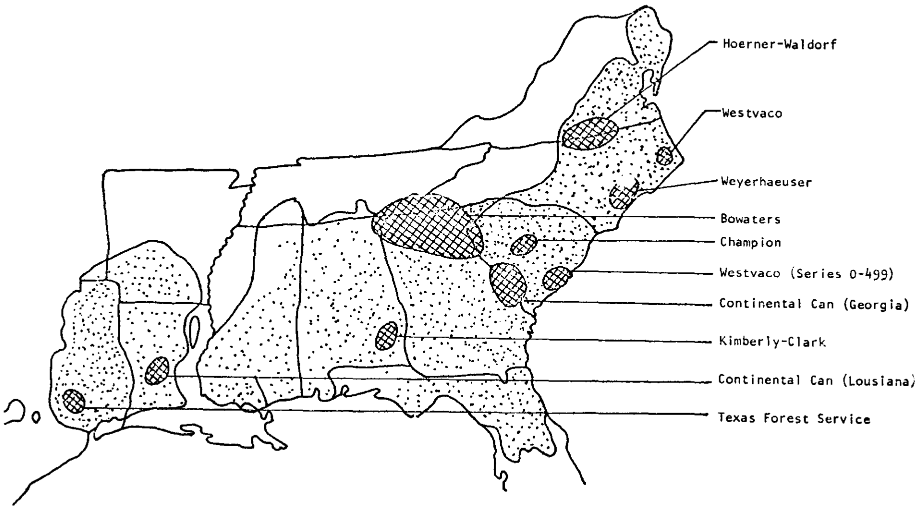

*Figure 1.* — Southeastern states showing natural distribution of loblolly pine and areas of origin parents used in the wide cross study.

## Data Analysis, Results and discussion

This is the first of a six-part series of reports that will examine various aspects of genotype × environment interaction and genotypic stability in loblolly pine based on data obtained from the wide cross study. The second part examines three genotypic stability parameters of potential use in tree breeding practice while the third part presents similar analyses of heterosis and heterosis × environment interaction. Part four concentrates on the correlation approach to analysis of genotype × environment interaction. The fifth part deals with the impact of the interaction on estimates of genetic variance components and on genetic gain predictions. In part 6 use is made of data obtained from the openpollinated progeny to draw some tentative inferences about the extent of genetic differentiation within the natural range of loblolly pine. Averages for all control pollinated and open-pollinated progeny at each location for

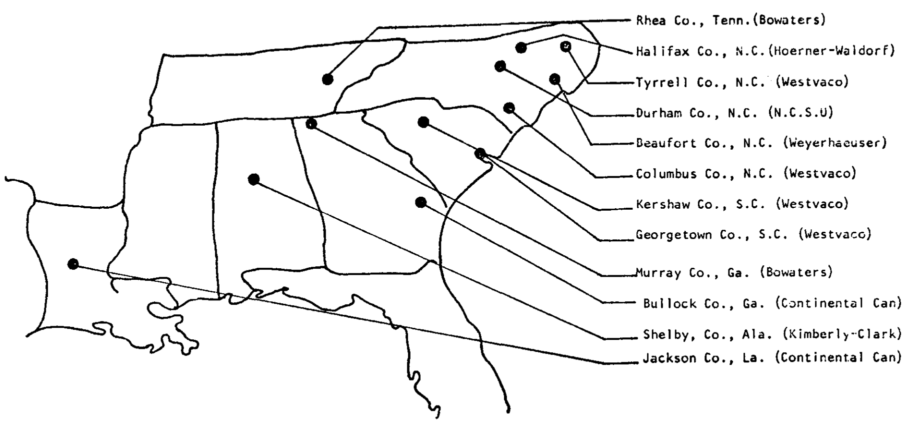

*Figure 2.* — Southeastern states showing test locations of the wide cross study.

*Table 2.* — Location, physical division, soil type, methods of site preparation, and number of replications for the wide cross study.

| County | State | Physical division | Soil type | Site preparation | No. of replications |
|---|---|---|---|---|---|
| Jackson | La. | Coastal plain | Sandy, well drained | Cleared of brush<br>No fertilization | 3 |
| Shelby | Ala. | Piedmont | Loamy texture cotton old-field | Cleared of brush<br>No fertilization | 2 |
| Bullock | Ga. | Coastal plain | Sandy, well drained | Clearcut and bedded<br>No fertilization | 3 |
| Rhea | Tenn. | Cumberland plateau | Sandstone and shale | Disked and windrowed<br>No fertilization | 4 |
| Halifax | N.C. | Coastal/Piedmont | Sandy with clayey subsoil. Natural drainage-poor | Clearcut and burned<br>No fertilization | 12 |
| Columbus | N.C. | Coastal plain (Green swamp) | Deep peat. Ditch drainage along the road | Cleared of brush<br>No fertilization | 4 |
| Murray | Ga. | Cumberland plateau | Sandstone and shale. Shallow hard pan in rep. 4 | Disked and windrowed<br>No fertilization | 4 |
| Kershaw | S.C. | Coastal/Piedmont (Sandhills) | Well drained deep, sandy soils | Clearcut and disked<br>Triple superphosphate at 25 lbs./acre | 3 |
| Tyrrell | N.C. | Coastal plain | Fine organic muck to 2-'. Then silt and tight packed sand | Clearcut and bedded<br>No fertilization | 10 |

height growth, percent fusiform rust infection, crown score and stem straightness score are shown on *table 3.* As expected, height growth was the trait most sensitive to environmental changes. The best height growth was over twice the poorest height growth. Such large differences are compatible with expectations because the test locations were purposely chosen to sample extreme environments as well as intermediate ones. There was also great variation in the percents fusiform rust infection from location to location but the incidence of the disease was not correlated with environmental site quality. This suggests the possible existence of different and localized pathogenic strains or differences in the number and density of local populations of the alternate host (Oaks) of the fungus. In contrast to height growth, there was little variation in crown score and stem straightness score. Although there was some indication that the trees had better crowns and straighter stems at the Bullock County, Ga. planting, the better form was most likely a reflection of scoring bias attributable to the scoring team at that location.

The data *(table 3)* indicate that meaningful results can only be gained using height growth for genotype × environment interaction and genotypic stability analyses and that other traits should only be used in certain special analyses. This recommendation will be followed in subsequent parts.

## Acknowledgements

Gratitude is expressed to members of the North Carolina State University Co-operative Tree Improvement Programme for their ready co-operation without which this study could not have been possible. Thanks also go to the graduate students who provided valuable assistance in field assessments.

## Summary

Suitable experiments for comprehensive analysis of the effects of genotype × environment interaction are rare in tree improvement programs throughout the world. The study reported herein was one of the most extensive of its type in the sense that it was appropriately designed to allow partition of the total genetic variance and also in the sense that many diverse environments were sampled.

The experimental material on which parts II to V (to follow) are based is outlined.

*Key words: Pinus taeda*, height growth, stem diameter, crown form, *Cronartium fusiforme.*

## Zusammenfassung

In diesem ersten von sechs Beiträgen werden Material, Kreuzungspläne, Feldversuchsanlagen und Datenerhebung

*Table 3.* — Average height (in feet), crown score, stem straightness score and percent fusiform fust infection for nine test locations.

| County | State | Avg. Height | % Rust infection | Avg. crown score | Avg. straightness score |
|---|---|---|---|---|---|
| Jackson | La. | 17.65 | 50.03 | 3.3 | 3.0 |
| Shelby | Ala. | 12.06 | 3.18 | 3.7 | 3.2 |
| Bullock | Ga. | 11.39 | 16.38 | 2.9 | 2.6 |
| Rhea | Tenn. | 11.11 | 3.96 | 3.4 | 3.4 |
| Halifax | N.C. | 10.61 | 3.88 | 3.5 | 3.5 |
| Columbus | N.C. | 10.49 | 15.76 | 3.8 | 3.2 |
| Murray | Ga. | 9.85 | 2.41 | 3.4 | 3.3 |
| Kershaw | S.C. | 8.27 | 14.84 | — | — |
| Tyrrell | N.C. | 7.79 | 1.47 | 3.5 | 3.8 |

eines 1968 groß angelegten Experiments zum Studium von Genotyp-Umwelt Interaktion und genotypischer Stabilität bei *Pinus taeda* behandelt. Der Versuch soll u. a. über geeignete genotypische Stabilitätsparameter, über Heterosis von Nachkommenschaften weit entfernter Eltern, die Verfälschung von Voraussetzungen des genetischen Gewinns durch Genotyp-Umwelt Interaktion und über die genotypische Stabilität von Nachkommenschaften frei abgeblühter Bäume und von Kreuzungen zwischen und innerhalb von Populationen Aufschluß geben.

### Literature Cited

Comstock, R. E. and Robinson, H. F.: The components of genetic variance in populations. Biometrics 4: 254—266 (1948). — Woessner, R. A.: A juvenile assessment of wide crosses of loblolly pine select trees indigenous to different geographic areas. Unpublished Ph. D. dissertation, Department of Forestry, North Carolina State University at Raleigh (1968). — Woessner, R. A.: Crossing among loblolly pines indigenous to different areas as a means of genetic improvement. Silvae Genet. 21 (1-2): 35—39 (1972).

---

# Genotype x Environment Interaction and Genotypic Stability in Loblolly pine

## II. Genotypic stability comparisons[*]

By F. Owino[1]

(Received Juli / September 1976)

### Introduction

Loblolly pine is the leading commercial forest species in the Southeastern United States. Its natural range extends from Maryland through the Carolinas to Georgia and northern Florida in the east and westward to east Texas. In recent years, there has been a marked trend of accelerated activity in forest production in this region with loblolly pine receiving much of the attention. It has become a stated objective of the large industrial forest land owners and the United States Forest Service that the projected accelerated regeneration be done only with genetically improved trees (Zobel 1975). This, together with the facts that (i) loblolly pine has a wide natural range, (ii) some of the forest land owners operate in large areas covering diverse environments, (iii) a very heavy exchange of research ideas and breeding materials has been instituted amongst the widespread membership of the co-operative tree improvement programs and (iv) intensive cultural practices of site preparation and fertilization are becoming more widely accepted, makes it imperative that the tree breeder ensure that selected genotypes have the widest adaptability along with superior volume production if he is to avoid 'genetic slippage' due to genotype × environment interactions. A practical handle on adaptability can be achieved through genotypic stability analyses. The investigation reported here was carried out to determine the stability of performance of groups of loblolly pine genotypes across several locations. The specific objectives of the investigation were:

1. To compare parameters of genotypic stability useful in tree breeding.
2. To compare the stability of wide crosses compared with local crosses.

### Literature Review

Ever since the introduction of the analysis of variance technique in the 1920's, plant breeders have reported statistically significant genotype × location, genotype × year or genotype × location × year interactions. For a long time such interactions were considered intractable and the routine has been to reduce them through experimental designs or to scale them out prior to analyses (Breese 1971). Experimental and theoretical studies of genotypic stability has advanced in two divergent directions: one followed by those interested in the underlying genetic mechanisms and the other followed by plant breeders seeking practical measures of stability.

The genetic approach can be traced to Lerner's (1954) development of the concept of genetic homeostasis. Grant (1963) made the distinction between phenotypic plasticity and physiological homeostasis. He pointed out that plants largely rely on phenotypic plasticity while animals rely on physiological homeostasis for their stability in changing environments. Of particular interest in this category are the studies of Jinks and Mather (1955) which showed that the extent to which a genotype interacts with the environ-

---

[*] This work was carried out in partial fullfilment of the requirements for the Ph. D. degree at North Carolina State University 1975.

[1] Lecturer, Botany Department, University of Nairobi, Kenya.