

Preliminary Testing of Eastern Cottonwood Clones

ROBERT E. FARMER, JR.,¹ and JAMES R. WILCOX²

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

Summary. Broad-sense heritabilities were 0.44 to 0.47 for height, 0.24 to 0.28 for diameter, 0.26 to 0.29 for volume, 0.69 to 0.70 for specific gravity, 0.36 for fiber length, and 0.83 to 0.89 for resistance to *Melampsora* rust in a breeding population of 100 eastern cottonwood clones from central Mississippi grown for 1 year on Commerce loam and Sharkey clay in 10 × 10 triple lattice designs.

The effect of site was statistically significant for growth and *Melampsora* rust, but not for wood properties. On the heterogeneous Commerce loam site, the lattice design was more efficient than randomized blocks for evaluating growth but not other characters. Both genetic and phenotypic correlations between characters were low and, with the exception of height × diameter ($r_p = 0.52$ to 0.65), of no practical significance in selection. Major variation in form was observed. Large-diameter cuttings elongated significantly faster than small ones through August, after which the effect of cutting diameter was not statistically significant.

Introduction

Eastern cottonwood (*Populus deltoides* Bartr.), an important forest tree species in the Mississippi Valley, is being bred to increase its genetic potential for good growth, form, wood properties (relatively high density and long fibers), and pest resistance (FARMER, 1966). Since cottonwood is propagated vegetatively on a commercial scale (MAISENHEDER, 1960), clonal testing is an important phase of the species' genetic improvement program (LIBBY, 1964; WILCOX and FARMER, 1967). Long-term testing of numerous clones is expensive; therefore it is desirable to screen clonal breeding populations formally prior to rotation-length tests. This screening includes brief tests to detect clones with relatively high early performance capabilities. The 1-year study of 100 clones reported here was designed to provide data on juvenile variation and inheritance and to develop methods of preliminary testing.

Methods

1. Material

Ninety-eight of the clones evaluated were from 2-year-old ortets that exhibited outstanding growth on Commerce loam in an open-pollinated progeny test of material from central Mississippi. The other two clones were from ortets of unknown parentage which had performed well in a nursery. Before they were tested, all clones were stored together in a nursery clone bank for one growing season. The population is believed to include a wide range of genotypes and to be typical of material available to the breeder in the Lower Mississippi Valley.

¹ Formerly stationed at the Southern Hardwoods Laboratory, which is maintained at Stoneville, Mississippi, by the Southern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, in cooperation with the Mississippi Agricultural Experiment Station and the Southern Hardwood Forest Research Group; presently plant physiologist, Division of Forestry Development, Tennessee Valley Authority, Norris, Tennessee.

² Formerly stationed at the Institute of Forest Genetics, Forest Service, U.S. Department of Agriculture, Gulfport, Mississippi; presently research geneticist, Crops Research Division, Agricultural Research Service, Purdue University, Lafayette, Indiana.

2. Site

Clones were tested for one growing season on two sites 3 miles apart in the Mississippi River flood plain. One site was recently cleared forest land. Its soil was a uniform Sharkey clay, which is dark and poorly drained with a montmorillonite clay content of 74–85% (BRUCE et al., 1958). Trees growing on Sharkey clay are subject to severe moisture stress in late summer; this soil is among the poorest being planted to cottonwood.

The second site was a forest nursery with a Commerce loam soil, which is one of the best soils in the Lower Mississippi Valley for growing cottonwood. The quality of the site varied somewhat due to scattered presence of clay beneath the plow layer.

3. Design and procedure

In February 1964, unrooted 50-cm-long stem cuttings of all clones were treated with a systemic insecticide (MORRIS, 1960) and planted in 10 × 10 triple lattice designs (COCHRAN and COX, 1957) on both sites. Cuttings were planted in three-ramet row plots at a 1.8 × 1.8 m square spacing. Mean cutting diameter by plot was approximately the same for all plots of a given clone, but varied from 7 to 20 mm among clones. Individual cutting diameters ranged from 5 to 30 mm. The plots were kept free of weeds until early fall by machine cultivation and hoeing.

4. Observations

In early May, before diameter increment was appreciable, diameters of all cuttings on the Commerce loam were measured. Total heights were measured on June 2, July 16, August 28, and October 27. Diameters of stems at 0.3 m above the soil surface were measured on October 29.

On November 2, before natural defoliation, plants were rated for their resistance to leaf rust (*Melampsora medusae* Thüm.) which infects plants around mid-August in the Lower Mississippi Valley. Leaves were sampled at breast height and rated according to the relative abundance of rust sori. A rating of 0 indicated that the tree was completely free of rust; a rating of 5, that 100% of the sampled leaf surface was rust infected and that some leaves were dying from infection.

Table 1. Summary of results

Character	Site	Test mean	Range of clone means	Genetic variance σ_g^2	Environmental variance σ_e^2	Heritability $\frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$	Predicted response to selection of top 10% of test population (% of mean)
Height m	Commerce loam	3.84	2.96	0.054153	0.068704	0.44	7
	Sharkey clay	3.51	2.90	0.050740	0.056290	0.47	8
Diameter, cm	Commerce loam	4.3	3.3	0.073839	0.191071	0.28	6
	Sharkey clay	4.1	2.8	0.059303	0.186838	0.24	5
Volume, m ³	Commerce loam	0.0021	0.0010	0.4168×10^{-6}	0.2791×10^{-6}	0.29	15
	Sharkey clay	0.0016	0.0008	0.0543×10^{-6}	0.1539×10^{-6}	0.26	13
Specific gravity, g/cc	Commerce loam	0.360	0.323	0.000271213	0.000118472	0.70	7
	Sharkey clay	0.361	0.318	0.000341556	0.000156367	0.69	7
Fiber length, mm	Commerce loam	0.84	0.71	0.00156154	0.00273011	0.36	5
	Sharkey clay	0.83	0.72	0.00125838	0.00227267	0.36	4
Rust rating*	Commerce loam	3.2	0.0	1.049924	0.211474	0.83	51
	Sharkey clay	3.7	0.0	1.053081	0.133651	0.89	46

* 0 = low rust infection.
5 = high rust infection.

For wood property determination, stem sections were taken 0.3 m above the soil surface from one tree in each plot on the Sharkey clay site and from two trees per plot on the Commerce loam. Specific gravity was determined from green volume and oven-dry weight of samples that varied in volume from 10 to 40 cc. Average fiber length was determined in samples taken adjacent to the cambium on one tree per plot. Two 1-mm-wide slivers were cut from the sample and macerated in a 1:1 mixture of glacial acetic acid and 30% hydrogen peroxide at 50 °C for 48 hours. The macerated, bleached fibers were washed in tapwater, stained for 15 minutes in Bismark brown solution and mounted in 1.5% agar solution on slides. The lengths of 50 whole fibers from each sample were then measured with a modified Ampliscope (WILCOX et al., 1964) at 50 ×, and an average was computed for each sample.

5. Analysis

Plot means for final height, diameter, volume, rust rating, and wood properties were analyzed by computer at the University of Georgia Computer Center, where a program for the 10 × 10 lattice design is currently on file. Since the 1-year-old cottonwood had a generally conic form, stems were assumed to be cones in volume computations. Thus, clonal variation in stem form, which was considerable, was not accounted for. Clone means were compared by Duncan's new multiple range test; least significant ranges were based on the average standard error of treatment means. Factors were tested at the 0.05 level of probability. The error and clone variance components essential to heritability computations were computed from the average effective error variance. Efficiency of the lattice design relative to randomized blocks was determined by comparing the error variance from a randomized block analysis with the effective error variance. (For a description of procedures see COCHRAN and COX, 1957).

Broad-sense heritabilities were calculated on an individual basis from the formula:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

where

σ_g^2 = total genetic variance

σ_e^2 = environmental variance.

Response to selection was computed from the calculated heritabilities with the formula $R = ih\sigma_G$ (FALCONER, 1960):

where

R = response to selection in terms of the unit of measurement

i = intensity of selection in terms of the standard deviation of the selected group, i.e., selection differential in standard measure

h = square root of the heritability

σ_G = total genetic standard deviation.

Phenotypic correlations among characters were computed from combined clone totals for the two sites. Genetic and phenotypic correlations based on covariance analyses were also calculated.

The relation between cutting diameter and height growth was investigated through correlation ana-

lyses and an analysis of cutting diameter effects in six clones.

Results

1. Growth

Mean clone height varied from 2.96 to 4.57 m on the Commerce loam site and from 2.90 to 4.24 m on Sharkey clay. Material growing on Commerce loam was taller than that on Sharkey clay; the clone-site interaction was not statistically significant. Data on first-year height and on the other characters observed are summarized in Table 1. The relative efficiency of the triple lattice design, compared to a randomized block design, was 198% on Commerce loam and 105% on Sharkey clay. The greater efficiency of the lattice on the Commerce loam site is attributed to the presence of a lens of clay which ran beneath part of one replication and drastically increased variation within that replication.

Broad-sense heritability for height was approximately the same on the two sites (0.44, Commerce; 0.47, Sharkey). About an 8% increase in mean height might be expected from selecting the best 10 clones in the test and using them directly by vegetative propagation.

Mean clone diameter ranged from 2.8 to 4.8 cm on Sharkey clay and from 3.3 to 5.6 on Commerce loam. As with height, the site effects were statistically significant, and the clone-site interaction was not. The relative efficiency of the lattice design on the Sharkey clay (104%) was approximately the same as for height on that site. Its efficiency for diameter on the Commerce loam site (162%) was less than for height, however. Heritability for diameter (0.28, Commerce; 0.24, Sharkey) was lower than for height, a feature observed in other tests (WILCOX and FARMER, 1967). Selection of the top 10 clones would result in a 5 or 6% increase in mean diameter.

Mean ramet volume was 0.0021 m³ for the Commerce loam and 0.0016 m³ for the Sharkey clay. Heritability for volume was approximately the same as for diameter, as was the relative efficiency of the lattice design. The predicted response to selection for volume, however, was greater than for either

height or diameter due to the wide variation in observed volumes (0.0008 to 0.0034 m³).

Because of its complex nature, no attempt was made to evaluate tree form quantitatively. However, most of the variation in form was interclonal. Figure 1 shows the observed range in tree form.

2. Wood properties

Mean specific gravity of clones varied from 0.32 to 0.41 g per cc, a range somewhat less than that observed in field sampling (FARMER and WILCOX, 1966a) and among individual trees in juvenile open-pollinated progenies (FARMER and WILCOX, 1966b). The mean for the test (0.36) was slightly below other observed means (FARMER and WILCOX, 1966a; WALTERS and BRUCKMANN, 1965). There was no difference in the specific gravity of the material on the two sites, and clone-site interactions were not statistically significant. The lattice design did not give appreciably greater efficiency than randomized blocks on either site. Broad-sense heritability on both sites was rather high (0.70). Selection of

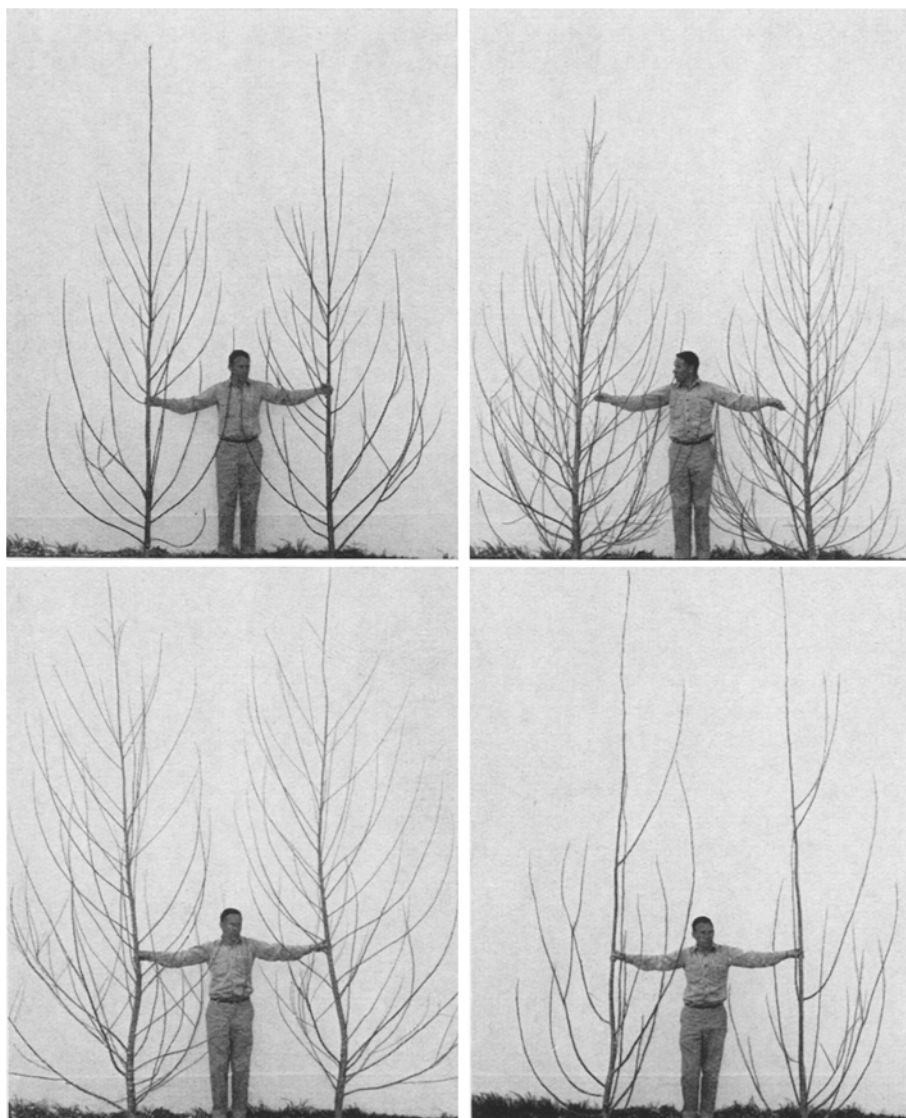


Fig. 1. Variation in juvenile form among four eastern cottonwood clones. Two ramets from a single clone are shown in each photograph. Note clonal variation in straightness and number and form of branches

Table 2. Phenotypic (r_p) and genetic (r_g) correlation coefficients based on covariance analyses

Relationship	Site	r_p	r_g
Height \times diameter	Commerce loam	0.65*	0.50
	Sharkey clay	0.52*	0.36
Height \times rust infection percent	Commerce loam	0.18	0.18
	Sharkey clay	0.30*	0.33
Diameter \times rust infection percent	Commerce loam	0.07	0.04
	Sharkey clay	0.01	± 0.00
Diameter \times specific gravity	Commerce loam	-0.21*	-0.32
	Sharkey clay	-0.16	-0.21
Diameter \times fiber length	Commerce loam	0.03	± 0.00
	Sharkey clay	0.14	± 0.00

* Statistically significant at the 0.05 level.

the top 10 clones would give a 7% increase in specific gravity over the test mean.

Clonal variation in fiber length was also about the same on both sites (0.71 to 1.00), but fiber length was less heritable (0.36) than wood specific gravity. One could expect a 4 to 5% increase in mean fiber length in response to selecting the best 10% of the tested clones. Clone-site interactions for this character were not statistically significant, and the lattice design effected little increase in test efficiency.

3. *Melampsora* rust resistance

Clone ratings varied from 0 to 5 on both sites, and distribution of ratings was roughly normal. Average rating for the trees on Sharkey clay was slightly (but significantly) higher than for those on the Commerce loam; clone-site interactions were not statistically significant. No increase in test efficiency due to the lattice design was observed. Heritabilities were high (0.83 to 0.89), and their application in selection response prediction indicated that mean rust ratings could be improved about 50% by selecting the test's best 10 clones. These data are similar to those of JOKELA (1966) for an Illinois clonal population.

4. Correlations

The relationships between growth rate and specific gravity, fiber length, and rust infection were investigated by covariance analyses; the resulting phenotypic and genetic correlation coefficients are presented in Table 2. While some of the correlations were statistically significant, except for height \times diameter, all were low and of little practical importance in selection.

5. Cutting-diameter: growth relationships

Correlations between mean cutting diameter of clones in May and mean clone height in June, July, August, and October were determined with data from the Commerce loam site:

Date	r
June 2	0.39**
July 16	0.28**
August 28	0.17
October 28	0.14

** Statistically significant at the 0.01 level.

A significant portion of clonal variation in height was thus accounted for by clonal variation in cutting diameter until July, when heights averaged 6 feet. Beyond that date the relationship was not statistically significant (0.01 level).

In a second analysis of cutting diameter effects, six clones were chosen which had equal mean cutting diameters in May.

Height growth of one large (19–23 mm) and one small (9–12 mm) cutting in each plot of these clones was observed throughout the season. The effects of these two cutting diameters on height at various dates were determined by split-plot analysis of variance. Growth of large cuttings was greater than that of small cuttings through the August measurement, at which time mean height was 11 feet. Cutting diameter effects were not statistically significant in October. Results of these two analyses indicate that nongenetic clonal effects ("C" effects of LERNER, 1958) associated with cutting diameter contributed significantly to variation in growth until mid- or late summer.

Discussion and Conclusions

Clonal variance in the test population was great enough to improve genetic potential for good early performance through selection. Broad-sense heritabilities for height, diameter, and *Melampsora* rust resistance were similar to those reported by WILCOX and FARMER (1967) for six replications of randomly selected cottonwood clones. Broad-sense heritability estimates for juvenile wood properties have not been previously published for cottonwood in the Lower Mississippi Valley. The greatest gains could be made in clonal selection for *Melampsora* rust resistance and tree volume. Although height and specific gravity were under stronger genetic control than volume, their smaller variance resulted in lower percentage gains from selection. The higher gain in tree volume than in either height or diameter can be attributed to greater variation in tree volume and to the positive correlations between tree height and diameter. The general lack of correlations between other characters indicates that gains in these traits will, for the most part, be made independently.

Lack of significant clone-site interaction suggests that preliminary testing and roguing can be done on a single site without appreciable loss of valuable clones, especially if selection is extensive. This conclusion is further substantiated by the fact that, except for height and diameter, site did not significantly affect the traits studied. These results do not mean, however, that long-term clonal testing on single sites is warranted. Further work with all stages of testing will be necessary before a definitive prescription for standard cottonwood clonal trials can be formulated.

On heterogeneous sites or sites of unknown variability, growth evaluation will be appreciably more

efficient with a lattice design than with randomized blocks. Given computer facilities, this advantage warrants the little extra work in design and layout. For preliminary tests, the triple lattice is particularly appropriate because a relatively large number of clones can be evaluated on a small area of land.

"C" effects hinging upon the relationship between cutting diameter and growth must be considered in preliminary testing until plants are well into their first growing season. Results of others (HOFFMANN, 1940; NEGISI et al., 1958) with *Populus* indicate that in areas where first-season growth is not as good as in the Lower Mississippi Valley, one must evaluate cutting diameter effects for more than one season. It is desirable to begin tests with cuttings of approximately equal size, even in Mississippi where rapid growth may mask the influence of cutting diameter by the end of the first growing season.

Zusammenfassung

Zur Auffindung von Klonen mit relativ frühzeitiger Ertragsleistung wurden Voruntersuchungen an Zuchtpopulationen von *Populus deltoides*, einer bedeutenden Waldbaumart im Mississippi-Tal, durchgeführt.

Der einjährige Anbau einer Zuchtpopulation von 100 Klonen aus Zentral-Mississippi in 10 × 10 Dreisatzgitter-Anlage auf Commerce-Lehm und Sharkey-Ton ergab folgende Heritabilitätskoeffizienten im weiteren Sinne für die untersuchten Eigenschaften: Höhe 0.44 bis 0.47, Durchmesser 0.24 bis 0.28, Umfang 0.26 bis 0.29, spezifisches Gewicht 0.69 bis 0.70, Faserlänge 0.36 und Resistenz gegen *Melampsora*-Rostbefall 0.83 bis 0.89.

Der Einfluß des Standortes erwies sich bezüglich Wachstum und Rostresistenz als signifikant verschieden, nicht dagegen bezüglich der Holzeigenschaften. Auf heterogenem Commerce-Lehm zeigte sich für die Bewertung des Wachstums die Gitteranlage geeigneter als die Blockanlage mit zufälliger Anordnung, nicht aber für die anderen Merkmale. Die genotypische und phänotypische Korrelation zwischen den Eigenschaften war gering und bei der Selektion von keiner praktischen Bedeutung mit Ausnahme der Korrelation zwischen Höhe und Durch-

messer ($r_p = 0.52$ bis 0.65). Eine größere Variabilität konnte bei der Wuchsform beobachtet werden. Stecklinge mit großem Durchmesser wuchsen im August signifikant schneller als solche mit kleinem, später war der Einfluß des Stecklingsdurchmessers nicht signifikant.

Literature

1. BRUCE, R. R., W. A. RANEY, W. M. BROADFOOT, and H. B. VANDERFORD: Physical, chemical and mineralogical characteristics of important Mississippi soils. Mississippi Agr. Exp. Sta. Tech. Bull. 45, 36 p. (1958). —
2. COCHRAN, W. G., and G. M. COX: Experimental designs, 2nd edition. New York, N. Y.: John Wiley and Sons 1957. —
3. FALCONER, D. S.: Introduction to quantitative genetics. New York, N. Y.: Ronald Press Co. 1960. —
4. FARMER, R. E., JR.: Cottonwood improvement in the Lower Mississippi Valley. Eighth Southern Forest Tree Impr. Conf. Proc., p. 49—52 (1966). —
5. FARMER, R. E., JR., and J. R. WILCOX: Specific gravity variation in a Lower Mississippi Valley cottonwood population. Tappi 49, 210—211 (1966a). —
6. FARMER, R. E. JR., and J. R. WILCOX: Variation in juvenile growth and wood properties in half-sib cottonwood families. Joint Proc. Second Genet. Workshop Soc. Amer. Forest. and the Seventh Lake States Forest Tree Impr. Conf. U. S. Forest Serv. Res. Pap. NC-6, p. 1—4 (1966b). —
7. HOFFMANN, R.: Die Abhängigkeit des Höhenwachstums der Pappelheister vom Durchmesser der Steckhölzer. Forstwissen. Cbl. 62, 20—21 (1940). —
8. JOKELA, J. J.: Incidence and heritability of *Melampsora rust* in *Populus deltoides* Bartr. In: Breeding Pest Resistant Trees, p. 111—117. New York, N. Y.: Pergamon Press 1966. —
9. LERNER, I. M.: The genetic basis of selection. New York, N. Y.: John Wiley and Sons 1958. —
10. LIBBY, W. J.: Clonal selection, and an alternative seed orchard scheme. Silvae Genet. 13, 32—40 (1964). —
11. MAISENHELDER, L. C.: Cottonwood plantations for southern bottomlands. U.S. Forest Serv. Southern Forest Exp. Sta. Occas. Pap. 179, 24 p. (1960). —
12. MORRIS, R. C.: Control of cottonwood insects with a systemic insecticide. J. Forest. 58, 818 (1960). —
13. NEGISI, K., K. YAGI, and T. SATOO: Studies in the growth of young plants of poplar arising from cuttings of different thickness. I. Seasonal course in dry weight increment. J. Jap. Forest. Soc. 40, 421—437 (1958). —
14. WALTERS, C. S., and G. BRUCKMANN: Variation in specific gravity of cottonwood as affected by tree sex and stand location. J. Forest. 63, 182—185 (1965). —
15. WILCOX, J. R., and R. E. FARMER, JR.: Variation and inheritance of juvenile characters of eastern cottonwood. Silvae Genet. (1967). In press. —
16. WILCOX, J. R., E. B. SNYDER, and J. P. MORROW: Modifications of the ampliscope for measuring hardwood fibers. J. Forest. 62, 581 (1964).