Variation and Inheritance of Eastern Cottonwood Growth and Wood Properties Under Two Soil Moisture Regimes

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Introduction

On sites assigned to intensive culture of eastern cottonwood (Populus deltoides BARTR.) in the lower Mississippi River flood plain, soil moisture availability is a major determinant of productivity. Variations in moisture characteristics of sites are usually related to soil texture, which ranges from sandy loams to heavy clays. Trees on sites of both textural extremes are commonly subjected to severe drought in late summer.

Cottonwood improvement programs in the region are designed to develop clones suitable for a variety of sites. Thus genotype-site interactions are major considerations in breeding. Differential response to moisture stress may be an important source of interaction variance.

This paper describes a pot study of genetic variation among 30 cottonwood clones growing under both favorable and stressful soil moisture regimes. The objectives were to determine how moisture stress may alter variation patterns and to provide information on variation in relationships between leaf, stem, and root growth.

Methods

In March 1966, 300 metal pots (51 cm wide, 46 cm high) were filled with approximately 0.07 cubic meter of Commerce loam and arranged outdoors in a nursery in five replications of 60 pots each. Each replication consisted of two rows of 30 pots. Soil surface was mulched with pea-sized gravel, and pots were drained by four 2.5 cm diameter holes near the base.

Unrooted stem cuttings (length, 13 cm; diameter, 4—10 mm) from 30 randomly selected clones were randomly assigned to pots in each of the ten 30-pot rows. On March 30, four cuttings were planted near the center of each pot with their tops flush with the surface of the mulch. After establishment, plants were thinned to one per pot.

Each of the two rows of pots in replications was randomly assigned to one of two soil moisture regimes. The first was a "control" regime of watering daily or twice daily to maintain near-optimum soil moisture conditions for growth. The second, a stress regime, was created by covering pots with waterproof covers and watering plants individually only after incipient wilt of the apex was observed at 8:00 AM; the soil was saturated when these plants were watered. Pot covers consisted of a hardware cloth frame covered with lightweight waterproof sacking. Plants grew through an 8-cm wide hole in this cover. Water was added to the pot through the hole.

Dates of rewatering were recorded for individual stressed plants. Control plants were thus growing in an almost continuously optimum soil moisture regime while stressed plants were subjected to drying cycles. Treatments were started on May 23, when plants were approximately 20 cm high (as measured from the surface of the mulch). During the test, 10 randomly selected plants in each of the two treatments were measured every third day to obtain a record of height-growth patterns.

Treatments were continued until September 9; at this time all plants were still foliated and making apical growth. Final measurements included total height, stem diameter at 8 cm above the soil surface, and dry weight of leaves, stems, and roots. Leaves were picked from plants, placed in paper bags, and dried at 50° C. Roots were washed free of soil with firehoses under moderate pressure. A negligible amount of root (<2.0 g oven-dry weight per plant) was lost in washing. While the greatest proportion of small roots was found within a 15-cm-wide area adjacent to pot sides and bottom, the root systems did not appear severely "pot-bound". Stems and roots were air-dried in a low-humidity greenhouse at approximately 38° C.

Specific gravity of 10-cm-long sections of stem taken at the base of plants was determined with the maximum-moisture technique (SMITH, 1954). Fiber samples were taken adjacent to the cambium from the same sections in three replications and mounted on glass slides by standard techniques (FARMER and WILCOX, 1966). Mean fiber length of samples was based on measurement of 50 whole fibers at 50X.

Combined analyses of data took the form of a split-plot design, and a randomized block design was used for separate analyses of data from the two treatments. Significance was tested at the 0.05 level of probability. Broad-sense heritability based on these analyses was computed as follows:

$$h^2 = \frac{\sigma^2_G}{\sigma^2_G + \sigma^2_e}$$

σ^2_G = genetic (clonal) variance
σ^2_e = environmental variance

Relationships between leaf weight and stem and root weights were investigated by covariance analyses, and by analyses of variance in ratios of stem and root weights to leaf weight, i. e., stem weight ÷ leaf weight.

Results

General Response to Moisture Stress

Typical patterns of growth for favorable and stress treatments are illustrated in *Figure* 1. The stressed plants were watered an average of four times during the test. The first drying period was 35 to 40 days, and apical growth had stopped at around 30 days. Later cycles were increasingly shorter as plants grew larger and depleted soil moisture more rapidly.

Clonal variation in wilting response was evaluated through an analysis of the number of days in the second drying cycle (late June to late July). Since some variation in plant size was apparent, an analysis of covariance was

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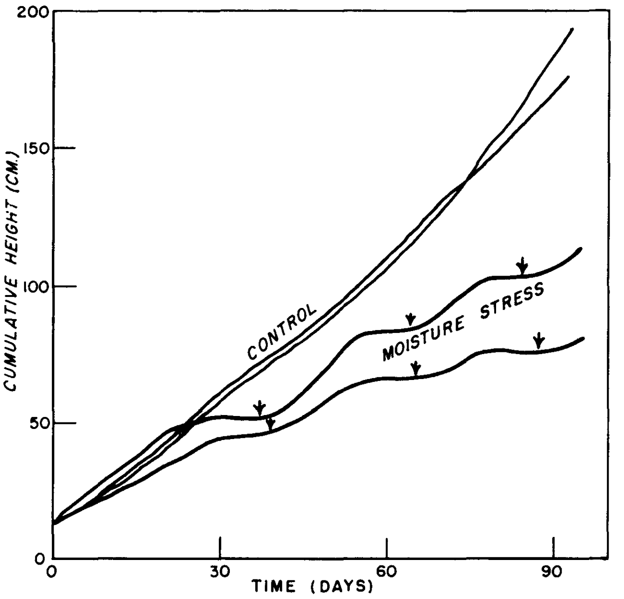

Figure 1. — Typical patterns of height growth under two soil moisture regimes. Arrows indicate dates on which plants were watered. Lines represent individual trees.

used with plant height on June 24 as the independent variable and days from watering to wilting as the dependent variable. Adjusted clone means ranged from 20 to 31 days *(Table 1)* and were significantly different. The broad-sense heritability of this parameter of drought resistance was 0.32.

Table 1. — Clonal variation in mean number of days (adjusted) from watering to wilting in second drying cycle. Means differing by three or more units are significantly different (DUNCAN's new multiple range test).

Clone number	Mean days
136	20
110, 124	23
132	24
105, 111, 130	25
121, 123, 133, 138, 139	26
112, 113, 117	27
104, 118, 120, 126, 134, 137	28
103, 107, 114, 115, 119, 128	29
106	30
108, 122	31

Major variation in type of reaction to moisture stress was informally observed among clones and appeared to account for the differences in clonal response. Following cessation of height increment, most of the stressed clones wilted after 5 to 10 days, during which time a few yellowed lower leaves were shed. However, plants of a few clones persisted without wilting for more than 2 weeks after growth cessation; most of the lower leaves yellowed and abscised before the plants wilted. At the other extreme, plants of some clones wilted severely only a few days after stopping apical growth. These plants lost very few lower leaves, since they were watered at wilting.

In all clones, apical growth resumed in 2 to 4 days after watering. During early cycles, stressed plants made apical growth as rapidly as those under the favorable treatment.

Growth

Variation due to treatment, clones, and the clone × treatment interaction was statistically significant for all characters *(Table 2)*. Plants under the favorable moisture regime made about twice the height and diameter growth of stressed plants, and their average dry weight was seven times greater (711 g vs. 103 g).

Pertinent components of variance computed from mean squares in split-plot analyses are presented in *Table 3*. The genotype × environment (clone × treatment) interaction variance, although usually less than genetic variance, accounted for a substantial portion of the variation in all growth parameters. For root weight, it was slightly greater than genetic variance. Major changes in clonal rankings in response to treatment are reflected in this interaction effect; rank correlation coefficients were all low (r = 0.20 to 0.33).

Table 2. — Summary of means, variances, and broad-sense heritabilities.

Character and moisture treatment	Mean	Range of clone means	Broad-sense heritability
Height, m			
Favorable	2.44	1.83—2.74	0.57
Stressful	1.16	0.82—1.43	0.40
Diameter, cm			
Favorable	2.9	2.4 —3.2	0.41
Stressful	1.3	1.2 —1.6	0.29
Foliage weight, gm			
Favorable	211	177—273	0.24
Stressful	35	23— 46	0.45
Stem weight, gm			
Favorable	259	196—316	0.29
Stressful	29	18— 41	0.43
Root weight, gm			
Favorable	241	151—324	0.24
Stressful	39	28— 57	0.40
Total weight, gm			
Favorable	711	595—812	0.13
Stressful	103	74—166	0.44
Shoot/root ratio			
Favorable	2.09	1.47—3.00	0.28
Stressful	1.63	1.28—1.97	0.15
Specific gravity			
Favorable	0.380	0.333—0.419	0.64
Stressful	0.337	0.295—0.386	0.76
Fiber length, mm			
Favorable	0.76	0.70—0.83	0.48
Stressful	0.62	0.55—0.69	0.43

Table 3. — Summary of variance components.

Character	Clones σ^2_G	Clones × treatment σ^2_{Gt}	Error σ^2_e
Height	0.0218	0.0048	0.0244
Diameter	0.0094	0.0050	0.0256
Foliage weight	114.11	31.17	398.56
Stem weight	168.50	121.35	686.82
Root weight	205.53	219.22	1,888.48
Total weight	657.92	320.10	5,329.81
Shoot/root	0.0353	0.0144	0.1347
Specific gravity	0.004719	0.000344	0.000204
Fiber length	0.000730	0.000708	0.000946

Broad-sense heritability of growth parameters varied from 0.13 for total dry weight in the favorable treatment to 0.57 for height under the same regime. Heritability estimates for height and diameter were larger under the favorable regime, but dry-weight heritabilities were all higher under stress.

Since rewatering followed observable wilting, drought-susceptible clones in the stress treatment received more water than drought-resistant ones. Hence, some of the growth variation under the stress regime was necessarily a result of the experimental procedure. This confounding may partly account for both the higher heritability of dry weight under the stress treatment and the appreciable genotype × environment interaction variance. Thus, while clonal variation in drought resistance was effectively delineated under the stress regime, the test procedure had limitations with respect to growth evaluation.

Shoot/Root Ratio

The relationship between shoot (leaf and stem) and root growth varied considerably from clone to clone *(Table 2)*. There is evidence that shoot/root ratios vary with plant age or total weight. The balance between the growth rate of shoot and root with increasing plant weight can be described by an empirical mathematical relationship referred to as the equation of allometric growth: shoot/root = aW^b, where a and b are constants and W is total plant weight (Huxley, 1932; Ledig and Perry, 1965). While an allometric relationship was not observed in this test, shoot/root ratio was related to plant weight: In the favorable treatment (shoot/root = 1.47 to 3.00) there was a negative correlation ($r = -0.56$) between mean clone weight and shoot/root ratio; the relationship was positive ($r = 0.42$) in the stress treatment (shoot/root = 1.28 to 1.97). Analyses of covariance were therefore used with shoot/root ratio as the dependent variable and total dry weight as the independent one. Variance components were computed from adjusted mean squares.

The combined covariance analysis revealed that, while effects of clones and the clone × treatment interaction were statistically significant, the treatment difference per se was nonsignificant after adjustment for plant size. The genotype × environment variance component was slightly less than half the magnitude of the genetic variance. Broad-sense heritability of the shoot/root ratio under favorable moisture conditions ($h^2 = 0.28$) was about the same as for leaf, stem, and root weight considered individually. Under soil moisture stress, heritability was lower ($h^2 = 0.15$).

Relationship of Leaf Weight to Stem and Root Weights

Phenotypic and genetic correlations (Falconer, 1960) based on covariance analyses were as follows:

	r_p	r_g
Favorable regime:		
Leaf weight × root weight	0.59*	0.49
Leaf weight × stem weight	0.38*	±0.00
Stressful regime:		
Leaf weight × root weight	0.79*	0.21
Leaf weight × stem weight	0.76*	0.73

* Significant at the 0.05 level.

In another approach, the ratios of leaf weight to stem and root weights were subjected to analyses of variance. Results are summarized below:

	Mean	Range of clone means	Broad-sense heritability
Favorable regime:			
Stem weight/leaf weight	1.24	0.77—1.50	0.61
Root weight/leaf weight	1.14	0.84—1.45	0.28
Stressful regime:			
Stem weight/leaf weight	0.88	0.60—1.45	0.28
Root weight/leaf weight	1.22	0.84—1.59	±0.00*

* Clonal variation nonsignificant.

Clonal variation in stem/leaf weight was especially notable in the favorable treatment, where the ratio had a broad-sense heritability of 0.61. This result is in agreement with the absence of a genetic correlation between leaf and stem weights. Under moisture stress, the ratio was smaller than in the favorable treatment, but the root-weight/leaf weight ratio was larger; the latter relationship was expected since shoot/root ratios were smaller under stress.

Wood Properties

In the favorable treatment, clonal means for specific gravity ranged from 0.33 to 0.42 *(Table 2)*. Under stress, specific gravity was lower (0.29 to 0.39). Effects of treatment, clones, and the clone × treatment interaction were all statistically significant. However, the interaction component of variance was relatively small *(Table 3)*; the rank-correlation coefficient of clone means for the two environments was 0.87, indicating little change in ranking. Broad-sense heritabilities were comparatively high ($h^2 = 0.64, 0.76$) in both environments. There was essentially no correlation between mean clone diameter growth and density ($r_p = 0.08$) within the favorable treatment.

Fiber length variation attributable to treatment and clones *(Table 2)* was statistically significant, but clone × treatment effects were not. Fibers were longer in plants under favorable conditions than in the smaller stressed plants, but there was no correlation between mean clone diameter and fiber length ($r_p = 0.06$) within treatments. Rank correlation of clone means for the two treatments ($r = 0.57$) was moderate. Broad-sense heritabilities under the two treatments were about the same ($h^2 = 0.48, 0.43$).

Discussion

Observed variation in juvenile drought resistance indicates that selection for this character should be successful. The small test population contained some clones which persisted 50 percent longer in drying cycles than others. The study did not, however, properly test performance of clones under drought.

While clonal variation in growth under the stressful regime was partly a procedural artifact, data from favorable regime represent natural variation patterns. Especially notable is that leaf, stem, and root weights had similar broad-sense heritabilities. Selection for leaf weight will be as effective as selection for stem or root weight. However, selection for one character will not necessarily result in a concomitant general increase in plant weight. The variance and correlation analyses both indicate that, under good growing conditions, relationships between leaf weight and stem and root weights may vary from clone to clone. Thus some clones (those with high stem-weight/leaf-weight ratios) appeared to be more efficient in assimilation than others. These clones all had high stem weights. Under stress, stem production per unit of leaf weight was lower than in the favorable treatment, and clonal differences in efficiency were less distinct, or, as in root/leaf ratio, nonexistent.

Furthermore, there were genetic differences in shoot/root ratio which were not simply reflections of plant size. It appears that the constants of the allometric formula may vary from clone to clone and must be determined individually and empirically. Because of this genetic variation, the use of stem data for predicting total weight or root weight of individual plants is risky unless formulae are devised for individual clones. For example, in this test the mean stem weight of the top three clones was 16 percent above test mean for stem weight; mean of the same three clones was only 3 percent above test mean for total weight and 8 percent below the test mean for root weight. Heritability of shoot/root ratio, which was about the same as heritability for growth, may serve as an estimate of the potential accuracy of predictions based on allometric formulae; i. e., in a population exhibiting high shoot/root heritability, the allometric formula for individual clones would be accurate.

Shoot/root ratios of plants under the stress regime were consistently smaller than those of the larger plants under the favorable regime. The same observation has been made in aspen and birch by Jarvis and Jarvis (1965). While covariance analysis indicated that this difference was related to plant weight, it is notable that the relationship is the reverse of what would be predicted from the allometric equation. Hence, the moisture stress regime would appear to be a drastic treatment under which the relative growth of shoots and roots is altered (Ledig and Perry, 1965).

The observed relationship between growth and wood properties is in agreement with current hypotheses. Specific gravity is positively correlated with the degree of cell wall thickening, which is in turn related to plant nutritional status (Larson, 1964) as well as to direct effects of moisture stress (Whitmore and Zahner, 1967). Then, genetic factors being equal, plants growing under optimum conditions would be expected to have higher specific gravity than those under moisture stress. Fiber length is dependent upon the length of cambial initials and upon intrusive growth during differentiation (Chalk, *et al.*, 1955; Hejnowicz and Hejnowicz, 1958). It is generally observed in *Populus* that length of cambial initials increases with successive cell generations, with consequent increase in fiber length with distance from pith. In this study, moisture stress probably reduced both the number of cell generations and intrusive growth.

Genetic variation in wood properties generally followed a pattern previously observed in field tests of juvenile cottonwood (Curlin, 1967; Farmer and Wilcox, 1968): Specific gravity was under relatively strong genetic control, fiber length had a moderate heritability, and genotype × environment interactions were of little practical significance. Thus, while general environmental effects are important with respect to wood properties in juvenile cottonwood, selection from formal tests under various moisture regimes should result in widely applicable genotypes.

Summary

In a pot study, 30 randomly selected cottonwood clones from central Mississippi were grown for one season under favorable and stressful soil moisture regimes.

Variation in drought resistance was under moderate genetic control. Genetic variance in leaf, stem, and root weight was statistically significant, as were genotype × environment interactions; some clonal variation under the moisture stress regime was probably related to watering procedure. Shoot/root ratios differed among clones in both regimes, as did production of stem and root per unit of final leaf weight. Moisture stress reduced both specific gravity and fiber length but had little effect upon clonal variation in these properties.

Literature Cited

Chalk, L., Marstrand, E. B., and Walsh, J. P. DeC.: Fiber length in storeyed hardwoods. Acta Bot. Neer. 4: 339—347 (1955). — Curlin, J. W.: Clonal differences in yield response of *Populus deltoides* to nitrogen fertilization. Soil Sci. Soc. Amer. Proc. 31: 276—280 (1967). — Falconer, D. S.: Introduction to quantitative genetics. New York: Ronald Press Co. 1960, 365 pp. — Farmer, R. E., Jr., and Wilcox, J. R.: Variation in juvenile growth and wood properties in half-sib cottonwood families. *In:* Joint Proc. Second Genetics Workshop of the Soc. Amer. Forest. and the Seventh Lake State Forest Tree Impr. Conf. 1965, pp. 1—4. Michigan State Univ. 1966. U. S. Forest Serv. Res. Pap. NC-6, 110 pp. North Central Forest Exp. Sta., St. Paul, Minn. — Farmer, R. E., Jr., and Wilcox, J. R.: Preliminary testing of eastern cottonwood clones. Theoretical and Applied Genetics 38: 197—201 (1968). — Hejnowicz, A., and Hejnowicz, Z.: Variations of length of vessel members and fibers in the trunk of *Populus tremula* L. Acta. Soc. Bot. Polon. 27: 131—159 (1958). — Huxley, J. S.: Problems of relative growth. London: Methuen and Co. 1932, 276 pp. — Jarvis, P. G., and Jarvis, M. S.: The water relations of tree seedlings. V. Growth and root respiration in relation to osmotic potential of the root medium (pp. 167—182). *In:* Water stress in plants. Slavik, Bohdan, ed., 1963, Prague. Czech. Acad. Sci., 1965. — Larson, P. R.: Some indirect effects of environment on wood formation (pp. 345—365). *In:* The formation of wood in forest trees. Zimmermann, M. H., ed., New York: Academic Press. — Ledig, F. T., and Perry, T. O.: Physiological genetics of the shoot/root ratio. Soc. Amer. Forest. Proc. 1965: 39—43 (1966). — Smith, D. M.: Maximum moisture content method for determining specific gravity of small wood samples. U. S. Forest Serv. Forest Prod. Lab. Rep. 2014, 8 pp. (1954). — Whitmore, F. W., and Zahner, R.: Evidence for a direct effect of water stress on tracheid cell wall metabolism in pine. Forest. Sci. 13: 397—400 (1967).