DROUGHT HARDINESS TESTS OF BLACK WALNUT SEEDLINGS AS RELATED TO FIELD PERFORMANCE

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Abstract.--Walnut seed was collected from trees at eight locations along the 38th parallel from central Kansas to eastern Kentucky. Trees were grown in the greenhouse, nursery, and on upland and bottomland field sites in southern Illinois. In the greenhouse, after the trees were nearly fully grown, we stopped watering them and observed the number of days to wilting. Trees from the east generally wilted soonest. Chlorophyll Stability Index (CSI), a measure of chlorophyll breakdown caused by heating, was measured from samples collected in the nursery. There was no east-west trend for CSI differences. The early laboratory and greenhouse tests were weakly related or not related at all to field performance of 4-year-old trees on two field sites.

Additional keywords: *Juglans nigra*, juvenile-mature, water stress, genetics, chlorophyll.

Early tests that differentiate among varieties of tree species at an early age can speed progress in tree improvement programs. Early drought hardiness tests might consist of laboratory or greenhouse tests on seed or seedlings, or simply growth evaluations at an early age in the nursery and field. To verify early tests of long-lived plants such as trees, it is necessary to correlate the early test data with data from the same varieties when they are older and grown under field conditions.

Although black walnut (*Juglans nigra* L.) grows best on deep, well-drained soils (generally coves, lower slopes, and floodplains), it is sometimes found naturally and planted on the usually drier and less productive upland sites. If we could find sources of walnut trees that would survive and grow well under dry conditions, we could recommend planting on these sites. It seems logical that walnut trees from a drier climate (central Kansas) might be better adapted to such sites than trees from wetter climates (eastern Kentucky). Although there has been no previous work with early test for drought hardiness in black walnut, there has been other encouraging work with other tree species (Kriebel 1963, Pharis 1966, Pharis and Ferrell 1966, Farmer 1970, Majumder 1970, Schultz and Wilhite 1969, and Seidel 1972).

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Our objectives were: (1) to determine if we could differentiate among geographic sources and families (progeny from a single tree) from diverse origins using laboratory and greenhouse tests thought to be indicative of drought hardiness, and (2) to correlate data from these early tests with survival and growth data from the same sources and families after 4 years in the field.

METHODS

In the fall of 1968, seeds were collected from trees at eight approximately equidistant locations along the 38° N parallel from central Kansas to eastern Kentucky. At each of six locations, seeds were collected from five trees. At each of two locations (one in western Missouri, the other in Illinois), seeds were collected from ten trees: five from an upland site and five from a nearby bottomland site. The seeds were cleaned, weighed and stored in a cooler over winter at 2° C, after which seeds from each parent tree were divided into two lots.

One lot was placed in a warm room until the seeds germinated. In May the germinated seeds were planted in stovepipe pots (15 cm x 61 cm) which were filled with sand and peat (1:1) and U.C. Fertilizer II (Baker 1957). These were placed in the greenhouse in a randomized complete block design (two trees per plot; three replications). When the seedlings were 25 cm tall, the cotyledons were cut off to reduce the effect of differences in seed size.

The seedlings were watered two times a week until mid-July, at which time watering was stopped. Then the trees were observed each morning so that we could record the number of days from when watering was stopped to when wilting occurred. "Wilting" was defined as the time when the majority of the leaflets were drooping and the tips of the leaves were beginning to turn brown.

Because of their larger leaf surface area, we expected some of the early germinators and/or rapid growers to wilt sooner than others. To adjust for this, we estimated the leaf surface area of each seedling, which was used as a covariate to obtain an adjusted "number of days to wilting." After the trees wilted, they were transferred to a shadehouse and watered regularly until mid-September, when they were measured to determine dry weight shoot/root ratios.

The second lot was sown in the nursery in a randomized complete block design, replicated three times, and used for chlorophyll stability measurements and field outplantings. In early September, chlorophyll stability was determined for three families in each of the eight sources. A complete analysis was run on each of four consecutive days, thus days were considered as replications. Mature leaflets were collected from trees in each family, from each of three blocks, placed in a plastic bag in an ice cooler, and brought immediately to the laboratory.

A 5-gram sample of leaflets from each family was placed in a beaker with 50 ml of distilled water and heated in a water bath at 58° C for 30 minutes. Then we added 150 ml of 80 percent acetone to the sample and ground it in a Waring blender for 2 minutes. Next, we filtered the sample to get a chlorophyll extract. To 2 ml of the filtrate we added 15 ml of acetone. This sample was then examined for light absorption at 660 mμ using a Spectronic 20 colorimeter. We used the same procedure for a 5-gram sample of unheated leaves. The difference between the readings from the heated and unheated samples was defined as the Chlorophyll Stability Index (CSI). Kaloyereas (1958) and Kaul and Roy (1967) have shown that CSI was useful in isolating drought hardy strains of loblolly pine and eucalyptus. Low CSI values suggest a high resistance to chlorophyll breakdown and consequently greater drought hardiness.

In the spring of 1970, the trees from the nursery were planted on an upland site and a bottomland site both in western Missouri and southern Illinois. Survival was so poor among all families after the first year in the Missouri planting that no further measurements were taken. Therefore, only Illinois field results are presented.

The bottomland site in Illinois has 40 to 50 cm of uniform topsoil (Wakeland silt loam), underlain with a slightly coarser material. This moderately well drained soil is considered a good site for growing walnut. However, some Wakeland silt loam soils are questionable for growing walnut because of poor internal drainage.

The upland site in Illinois is on a south and west facing slope with a badly eroded Hosmer silt loam soil having a fragipan at a depth of 15 to 25 cm. We considered this a poor (unfertile and droughty) site for walnut (Losche _et al_. 1972). A randomized block design with three replications was used for each planting. The four trees in each linear plot were planted 12 feet apart.

RESULTS AND DISCUSSION

PLANTATION PERFORMANCE

Trees on the bottomland site averaged 113 cm tall after four years in the field compared with 57 cm for trees on the upland. Survival was 82 percent on the bottomland site and 47 percent on the upland. Survival among families ranged from 33 to 100 percent on the bottomland and from 8 to 92 percent on the upland site.

Trees from sources both east and west of Illinois apparently grew and survived about the same when planted on these two sites (table 1). There was no trend for longitude of seed source with survival, height, and diameter of trees. The linear regression coefficients were not significant at the 0.05 level.

Table 1.--Number of days to wilting, growth and survival for eight geographic sources of black walnut

Source	Longitude	No. days to wilting in greenhouse	Bottomland site: Ht.	Bottomland site: Dia.	Bottomland site: Survival	Upland site: Ht.	Upland site: Dia.	Upland site: Survival
	°W		cm	mm	percent	cm	mm	percent
KA	97.5	33	73	17	72	37	9	46
KA	95.5	29	113	20	50	49	10	40
MO	93.1	28	125	23	91	64	10	38
MO	91.3	27	122	24	67	55	11	37
IL	89.4	25	110	20	81	55	8	66
IN	86.6	26	119	21	92	52	9	62
KY	84.5	28	113	23	88	46	9	41
KY	82.7	22	101	20	95	49	9	35

Trees that were tallest on the upland site tended to be tallest on the bottomland. The correlation coefficient was 0.55 and significant. Among the tallest 15 families on one site, 9 were among the 15 tallest families on the other. However, the correlations between sites for survival or diameter were not significant.

Trees grown from seed collected from sources growing on upland sites in Missouri and Illinois generally survived and grew as well as those grown from seed collected from sources from adjacent bottomlands (table 2). It appears that the upland and bottomland populations of black walnut are not distinct at these Missouri and Illinois locations. Based on this small sample, it seems reasonable to suggest that no special attention need be given to site when collecting seed or making plus-tree selections. Although there were distinct differences in site quality, elevational differences were relatively minor (a few hundred feet). Greater elevational differences might well have produced different results. For example, Townsend and Roberts (1973) suggest that different water stress response exists for red maple (*Acer rubrum* L.) from dry and wet sites.

Table 2.--Growth and survival of black walnut trees from two sources when grown on two sites in southern Illinois

Sources	Plantation location					
	Upland site			Bottomland site		
	Ht.	Dia.	Survival	Ht.	Dia.	Survival
	cm	mm	percent	cm	mm	percent
Uplands:						
Missouri	74	12	40	132	24	95
Illinois	54	9	60	102	19	77
Bottomlands:						
Missouri	54	10	36	119	21	87
Illinois	57	10	75	123	22	86

CHLOROPHYLL STABILITY INDEX

There was no east-west trend for Chlorophyll Stability Indices. An analysis of variance did show significant differences among families (over all sources), but Tukey's test revealed that only the two lowest families differed from the two highest families.

When we compared these Indices for families with the number of days to wilting in the greenhouse and with the height, diameter, and survival data, none of the correlation coefficients were statistically significant at the 0.05 level. Therefore, it appears that CSI is not a useful procedure for predicting which walnut families will have good survival and rapid growth.

We can't explain why we got so few significant differences and no trends for the CSI data. If we refined the techniques we might expect more differences. And yet in our preliminary work, we used various modifications of the procedure and there was no indication that we would get a lot of differences. We also used the pressure bomb to measure the internal water stress in walnut leaves and found no differences among families (Scholander et al. 1965). If genetic differences for these traits do exist, they were masked by the laboratory technique that we used and/or by the microenvironmental influence in the field.

GREENHOUSE WILTING

Trees from sources in the western areas of the range did not wilt as quickly as trees from sources farther east (table 1). There was a significant linear trend for number of days to wilting with longitude of seed source. The regression coefficient was significant at the 0.01 level. However, on a source basis the number of days to wilting was not correlated with survival, height, or diameter.

On a family basis (over all sources), trees that wilted soonest also had slightly better survival on both upland and bottomland plots; r = -0.32 and -0.38. Number of days to wilting was not correlated with height and diameter. When we adjusted the family means for source effects, we found that on the bottomland site diameter was significantly correlated with number of days to wilting; whereas, on the upland site, height, diameter, and survival were all significantly correlated with number of days to wilting. The correlations were all negative and significant at the 0.05 level.

Even though the relationships are definitely weak, the "number of days to wilting" trait might be useful for estimating field performance on upland sites. It is surprising to see that the relationship is negative; trees that wilt soonest survive best and are the largest. We tried to account for size differences by adjusting the "number of days to wilting" using leaf surface area at the time we stopped watering as the covariate. We know that the trees having the greatest leaf surface area at the time we stopped watering were also larger for at least some time previous to that time. Perhaps cumulative growth should have been taken into account in our adjustment. Another possible explanation is that wilting is a defense mechanism that, for some reason or other, benefits the tree. In a teleological sense, we might say that the tree "knows" that it has a better chance of surviving if it wilts under water stress. We did not measure soil moisture at time of wilting, so we could not determine if wilting occurred at different moisture levels for different trees. Additional studies would be necessary to establish cause and effect relationships.

On a source basis, there was no trend for dry weight shoot/root (S/R) ratio with the longitude of the seed source. When analyzed on a family basis, the correlations between S/R ratio and survival, height, or diameter were not significant. However, trees in sources having the highest S/R ratios wilted soonest. The correlation coefficient of -0.38 was highly significant. On a family basis, the correlation between the same variables was -0.41, which also was highly significant. These were not typical nursery-grown seedlings on which the S/R ratios were computed. The seedlings were grown in pots in the greenhouse under a water stress until they wilted and then watered and transferred to a shadehouse. Under these conditions, we can say that S/R ratio is not a good predictor of field performance.

On the basis of this study, it appears that we cannot be optimistic about predicting field performance of young black walnut trees using laboratory and greenhouse drought hardiness tests. For example, the "number of days to wilting" trait followed an east-west trend, but the field growth results did not. We don't wish to preclude that these techniques can't be refined or that new techniques can't be developed that yield some good juvenile-mature correlations. At this time, however, it looks like there is no substitute for plantation evaluation for differentiating among sources and families of black walnut.

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