GEOGRAPHIC VARIATION IN SEED AND SEEDLING CHARACTERISTICS OF BLACK CHERRY (PRUNUS SEROTINA EHRH.)[1]

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Black cherry (Prunus serotina Ehrh.) is one of the most valuable timber species of the Appalachian Region. The stability and excellent working qualities of its wood make it highly desirable for cabinet and furniture manufacturing.

The natural range of this species is extensive, including most of the eastern portion of the United States, extending west into Nebraska, Kansas, and Oklahoma. Black cherry does not occur in the southern portions of Louisiana and Florida. Varieties and subspecies of Prunus serotina occur as far west as Arizona, and south through Mexico, into Guatemala (Hough 1960).

The commercial range of black cherry is limited to portions of Pennsylvania, New York, Maryland, West Virginia and Ohio. Other states producing small amounts of commercial black cherry are Michigan, Indiana, Virginia, Kentucky, Tennessee and North Carolina (Hough 1960). The fact that the commercial range of black cherry is restricted emphasizes the species importance in areas where merchantable size is attained.

When a plant species occurs over a wide geographic range, but reaches optimum development in only a limited portion of this range, it is evident that considerable geographic variation must exist within the species.

Two limited provenance studies of black cherry have been recently established. The first was designed "to study the genetic properties of black cherry native to different locations in Maryland" (Genys 1963). The second was initiated by the TVA and has the objectives of determining (a) whether trees from high elevation seed sources will grow well at low elevations, and (b) whether trees from low elevation northern seed sources are different from those from high elevation southern seed sources (Taft 1965).

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In 1966, a comprehensive study of black cherry was initiated at West Virginia University to determine (a) if the existing range-wide geographic variation (phenotypic variation associated with location) is, in fact, racial variation (genotypic variation associated with location), and (b) which is the best seed source, or sources, of black cherry for planting in the commercial range in West Virginia. This report presents preliminary results concerning the magnitude and nature of geographic variation in seed and seedling traits of black cherry.

Seed collections were obtained with the aid of cooperators from 33 widely separated geographic areas which represent the greater part of the natural range of black cherry and are well distributed (fig. 1). Major emphasis was placed on collections from sources within the Appalachian Region.

Figure 1.--Location of seed collections

The seed collections made in Alabama, Georgia, Florida and South Carolina apparently were within the range of *Prunus serotina* subspecies *hirsuta* (McVaugh 1951); however, the majority of seed came from areas populated by *Prunus serotina* var. *serotina*.

At each source, seeds were collected from five mother trees which were growing at least 150 feet apart. No restrictions were placed on site or tree quality, although adequate descriptions of these factors were requested. Seeds were depulped by hand and air-dried for three or four days in air-conditioned rooms. This process yielded clean seed with approximately equal moisture content for the purpose of obtaining comparative measurements of seed characters.

Germination tests were made in greenhouses and under natural environmental conditions in the Forestry Division Nursery near Morgantown. All seed was pretreated by soaking for 48 hours in a 0.1 percent citric acid solution to increase total germination and decrease time required to reach 90 percent of total germination (Jones 1963).

Following citric acid treatment, seed to be planted in the greenhouse was stratified for 90 days in moist sand at 34° - 38° F. It was planted on April 5, 1967, in a randomized, complete-block design with four replications (two in each of two greenhouses). Seedbeds in the nursery were fumigated with "Triazone" two weeks before planting. They were seeded in November, 1966, in a randomized complete-block design with five replications.

Germination began on April 7 in the greenhouses and was essentially completed by June 15, while germination in the nursery began on March 30, and was essentially complete by May 30. Weekly counts were made in each test to determine speed of germination.

Seed weights representative of each parent tree were obtained by weighing three random samples composed of 100 seeds each. A five-seed sample was drawn randomly from each parent tree collection and was measured to determine variation in seed diameter and seed-coat thickness. A vernier micrometer with a pressure-sensitive adjustment was used to determine seed-coat thickness. The micrometer face placed on the concave side of the seed coat was modified to a rounded point in order to increase measurement accuracy. Seed-coat thickness was measured to the nearest 0.001 inch.

Total height of each seedling in the nursery was measured on July 3, 1967, to the nearest centimeter.

Data were analyzed by analysis of variance, correlation analysis and multiple comparison procedures. A nested classification for a random-effects statistical model was used, providing estimates of the following variance components: geographic location, trees within location, and seeds within trees within location. Genetic correlation analysis was used to determine the relationship between various seed and seedling characters. For each trait, the degree of variation associated with the variance components was expressed in percent of the total (excluding the "replication" component in the progeny data).

RESULTS AND DISCUSSION

Parental Data

Seed Weight.--Variations in average seed weights among mother-trees ranged from 3.29 to 12.71 gms. per 100 seed. Average seed weights among seed sources ranged from 4.23 to 11.54 gms. per 100 seed. Most variation (69 percent, was associated with seed source, and variation among mother trees accounted for the remaining 31 percent. Variation among seeds within individual trees was less than 1 percent (table 1).

Seed Diameter.--Average seed diameters of all mother-trees ranged from 0.35 to 0.56 cms., while mean seed diameters for seed sources ranged from 0.39 to 0.53 ems. The majority of the variation was due to geographic location (64 percent) as indicated in table 1.

Seed-Coat Thickness.--Mean seed-coat thickness ranged from 0.007 to 0.027 among all mother trees and from 0.011 to 0.023 among seed sources. Seed origin accounted for 61 percent of the variation and mother-trees and individual seeds accounted for 29 and 10 percent, respectively, of the total variance (table 1).

Table 1.--Mean squares and estimates of variance components

Source of variation	Seed weight	Seed diameter	Seed-coat thickness
		Mean squares	
Locations	60.2236**	0.0502**	0.0003**
Trees: Loc.	5.4444**	0.0039**	0.00003**
Seeds: Trees	0.0222	0.0005	0.000002
	Estimated components of variance - percent		
Locations	69	64	61
Trees: Loc.	31	21	29
Seeds: Trees	0	15	10

** Significant at the 1 percent level.

Correlations and Similarities Between Seed Characters.--There were highly significant positive correlations among seed characters (table 2). A source which produced heavy seeds generally produced seeds of large diameter and thick endocarp. Trees within a location which produced the heaviest seeds generally produced seeds having the largest diameter and thickest endocarp.

Table 2.--Genetic correlations among seed and seedling traits in black cherry

Characters to which correlation applies		Components to which correlation applies: Seed sources	Within-seed sources: Trees:Sources	Within-seed sources	Within-seed sources: Seed:Trees
(1) Seed weight	(2) Seed diameter	.942**		.814**	
	(3) Seed-coat thickness	.819**		.706**	
	(4) Germinability (nursery)	.167		-.198*	
	(5) Germinability (greenhouse)	.003		-.225*	
	(6) Height (July)	.475**		.261**	
(2) Seed diameter	(3) Seed-coat thickness	.594	.562**		.097
	(4) Germinability (nursery)	.291		-.293**	
	(5) Germinability (greenhouse)	-.007		-.272**	
	(6) Height (July)	.340*		.240*	
(3) Seed-coat thickness	(4) Germinability (nursery)	.359*		-.198*	
	(5) Germinability (greenhouse)	.175		-.154	
	(6) Height (July)	.279		.128	
(4) Germinability (nursery)	(5) Germinability (greenhouse)	.596**		.638**	
	(6) Height (July)	-.146	.539**		-.085

* Value of r significant at 5 percent level. ** Value of r significant at 1 percent level.

Sixty to seventy percent of the total variation in seed characters was associated with geographic location; 20-30 percent was associated with mother trees within locations; and 1-15 percent was associated with seeds within trees. The consistent proportional distribution of variance components for each trait suggests that these traits are influenced by similar environmental and/or genetic controls.

The pattern of variation (table 3) for seed traits is random throughout most of the black cherry range. However, seed from the southern and southwestern portions of the range were characteristically lighter in weight, smaller in diameter and had thinner endocarps than those obtained from other areas (fig. 2).

Table 3.--Ordered arrays of seed source means and indications of significant differences among means at the .05 level

Seed weight - LSD .05 = 1.779		Seed diameter - LSD .05 = 0.037	
T-2	11.541 gms	T-2	.529 cms
P-1	11.077	WV-3	.527
NC-1	10.241	WV-1	.524
V-3	10.130	WV-5	.518
T-1	10.063	P-1	.516
WV-1	9.956	C-1	.516
W-1	9.816	NC-1	.514
VT-1	9.593	P-2	.506
C-1	9.481	W-1	.504
F-2	9.476	W-2	.504
WV-3	9.457	P-3	.503
W-2	9.117	VT-1	.502
WV-5	8.959	P-4	.502
NY-1	8.901	V-1	.500
P-3	8.871	NY-1	.499
MD-1	8.847	V-3	.496
P-2	8.776	T-1	.495
V-1	8.769	M-1	.486
P-4	8.743	WV-4	.481
M-1	8.371	MD-1	.479
WV-4	7.989	WV-2	.476
V-2	7.450	F-2	.469
WV-2	7.342	V-2	.458
F-1	6.565	F-1	.452
G-1	6.403	G-1	.452
L-1	5.932	L-1	.414
T-3	5.417	T-3	.411
AL-2	5.229	AL-2	.406
TX-1	4.719	AL-1	.398
AL-1	4.501	TX-1	.392
SC-1	4.235	MS-2	.392
MS-1	4.230	SC-1	.389
MS-2	4.230	MS-1	.384

AL = Alabama	MD = Maryland	T = Tennessee
C = Canada	MS = Mississippi	TX = Texas
F = Florida	NC = North Carolina	V = Virginia
G = Georgia	NY = New York	VT = Vermont
L = Louisiana	P = Pennsylvania	W = Wisconsin
M = Michigan	SC = South Carolina	WV = West Virginia

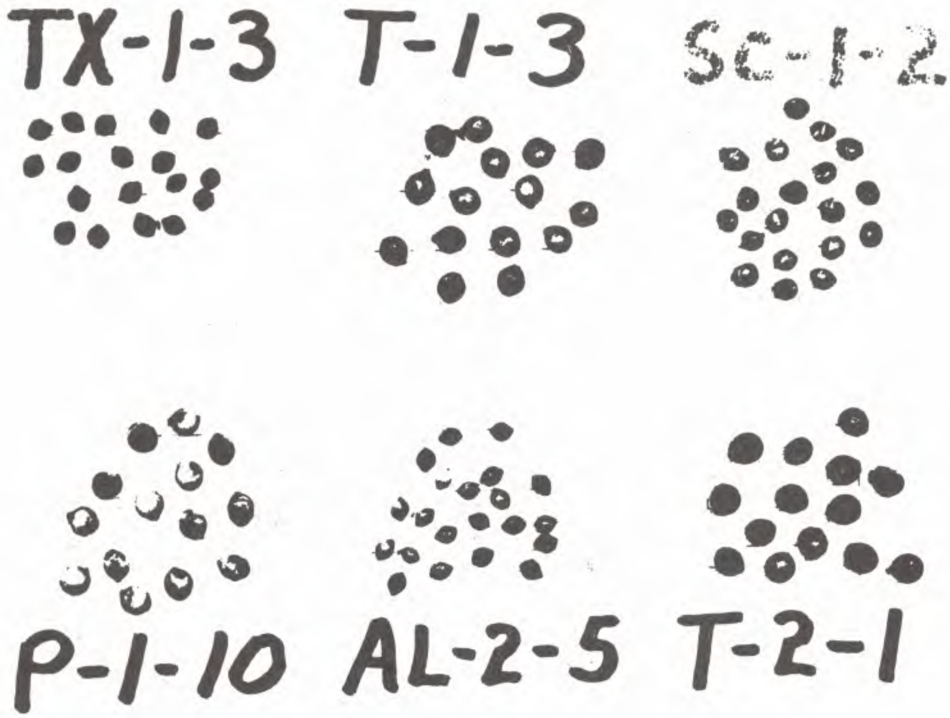

Figure 2.--Cherry seed from Texas (TX-1-3), Tennessee (T-1-3: T-2-1), South Carolina (SC-1-2), Pennsylvania (P-1-10), Alabama (AL-2-5)

Progeny Data

Germination.--Germination in the greenhouses ranged from 0 to 97 percent for mother trees, and from 0 to 91 for seed sources. Germination in the nursery also varied greatly, ranging from 0 to 94 percent for mother trees and 0 to 82 percent for seed sources. The Georgia seed exhibited the greatest germination in both tests. One Florida seed source did not germinate in either test, apparently due to improper seed handling. Geographic locations and mother trees contributed approximately equally to the variability in germination performance in both tests (table 4).

Table 4.--Mean squares and estimates of variance components obtained from analyses of variance of progeny data

Source of variation	Nursery germination	Greenhouse germination	Total height
	Mean squares		
Replications	215.1866**	74.1820**	260.1255**
Locations	300.1559**	589.1880**	113.2581**
Trees: Loc.	78.3659**	123.8152**	18.7561**
Error	7.4940	7.0883	8.6532
	Estimated components of variance - percent		
Locations	31	42	28
Trees: Loc.	45	47	14
Error	24	11	58

** Significant at the 1 percent level.

Differences in speed of germination among seed sources were largely random in nature. The period of time from planting to first germination was highly variable among mother trees, with those germinating earliest generally having greatest total germination.

Seedling Appearance.--For several weeks after nursery germination, seedlings of souhrn and southwestern origin were easily recognized on the basis of leaf color, leaf size, and shoot color. These sources had reddish-green immature leaves, red shoots and small primary leaves. Seedlings of the Florida, south Georgia and southern Alabama origins had leaves which, upon maturing, were more shiny and darker green than those from other origins. These characteristics of southern-origin seedlings were observed also under greenhouse conditions, but they were not as distinct.

Height Growth.--Means for height growth by July 3 showed extreme variation among mother trees 5 to 16 cms.) and among seed sources (5 to 12 cms.). Seedlings of Vermont, northeast Tennessee (T-l), and North Carolina origins were the tallest, while those of the Alabama and Canadian origins were the shortest. Only thirty percent of the variance was accounted for by seed source and a large proportion (57 percent) could not be accounted for by the variables measured. Much of this may have been due to time of germination and seed size, since seed weight was closely and positively correlated with seedling height at 3 months. A strong positive correlation was found. between 3-month height and total nursery germination.

Although the differences among provenances in mid-season height were highly significant, no definite geographic trends were apparent.

Summary and Conclusion

The primary purpose of this study was to determine the magnitude and nature of geographic variation associated with seed and seedling characteristics of black cherry. Three morphological and three physiological traits were studied in parent and progeny material representing 33 seed sources and a total of 149 mother trees.

Since the study is in its initial stages, a complete picture of geographic variation in black cherry cannot be presented here; however, a few definite conclusions can be drawn:

1. All traits studied showed highly significant differences associated with geographic sources. Variation in seed traits was strongly associated with geographic location (60 to 70 percent). Individual trees and seeds-within-trees contributed very little to overall variation. On the other hand, variation in germination was about equally accounted for by geographic locations and mother-trees-within-locations. The effect of geographic location was nearly three times greater for height growth at three months than that due to tree-to-tree effect; however, much of the variation in growth could not be accounted for by factors studied.

2. Seed from geographic locations characterized by heavy seeds also had characteristically thicker seed-coats and larger diameter; seedlings from these sources also attained the greatest total height at three months. On the average, trees which had th greatest average germination performance also produced the tallest seedlings.

3. Patterns of geographic variation for those progeny traits studied were largely random. Seed sources from south of 36° N. latitude generally exhibited similarities in seed. characteristics. However, there were no definite geographic patterns for simi larities and/or differences of sources from central and northern latitudes.

LITERATURE CITED

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DISCUSSION

JAYNES - Jerry, I have a question concerning the self-incompatibility of the cherry. What assurance do you have that the mere bagging is not preventing seed development? You may be getting fertilization, but because of the humidity change or other factors that you're imposing on these flowers their development may be prevented. We run into this with other tree groups, certainly in chestnut, we have individual trees which, as soon as you put a bag on them, they drop the flowers.

STAIRS - I do not have any real assurance of this since we only isolated the flowers and did not use control pollinated controls. I believe a more intensive study of controlled pollination in black cherry is in progress at the University of Florida under the direction of Dr. Ray Goddard. Perhaps they will be able to soon provide more definite answers to this question.

JAYNES - I just wondered If you had a few control crosses in addition to the selfs.

STAIRS - No, we didn't attempt controlled crossing in our research scheme, and that is certainly a valid criticism of our results.

KRIEBEL - I was also going to ask Jerry about the possibilities of apomixis. Did you find any evidence of this at all?

STAIRS - No; we did conduct cytological evaluations on approximately 100 developing embryo-sacs and found only degeneration in the self-pollinated material. This is too small a sampling to rule out the possibility of apomixis, although the total isolation study indicates that parthenogenesis is certainly rare if it occurs in this species.

KRIEBEL - I wanted to say something about the last paper. I was struck with the similarities to the situation in the southern sugar maple, which has a smaller leaf, more blunt points on the leaves and smaller seeds. We found in the nursery the first year or two that the growth was greater in the southern maple, but then as time vent by, this tended to be diversified along the lateral branches, so that later on the other trees overtook them. Did you find any evidence of lateral branching in your southern trees?

KITZMILLER - Yes, many seedlings are beginning to develop numerous lateral branches.

KRIEBEL - More in the southern trees?

KITZMILLER - Yes, the northern sources exhibit much stronger apical dominance than the southern sources.

KRIEBEL - What did the mature southern trees look like; were they more bushy than the northern ones? Do you know?

KITZMILLER - I don't know. Perhaps Dr. Cech would like to make a comment.

CECH - In answer to your question, Howard, according to the notes we received from most of our cooperators, many of the trees from which they made collections were located in a yard or along a hedge raw somewhere, so that I would imagine that most of them were bushier specimens, more open grown, and of smaller diameter than trees from which seed were collected up here in the north.

GENYS - May I break in at this time to mention to Dr. Cech that I have produced a note on my black cherry studies in Maryland. This includes information on the behavior of eight different strains in the nursery. Since very little is published on this subject, this note may be worth including in literature review.

DORN - A question for Jerry Stairs. Did I understand you correctly to say that within a single tree the anthers mature approximately the same time that the stigmas were receptive?

STAIRS - Yes, the anthers of a single flower seem to mature at the time of stigma receptivity. There is variation within the raceme, and also tree-to-tree and stand-to-stand variation, but within a single flower it appears to be synchronous.

FECHNER - I'd like to direct a question to Jerry. On these bagged flowers would you assume they were selfed? You didn't control selfing. You said that you got a bit of seed set, but no viable seed, is that correct?

STAIRS - Yes, we collected four seeds, but none of them germinated. They really should have been evaluated by X-ray technique prior to sowing, but we failed to do this.

FECHNER - Did most of the flowers abscise, or did the racemes abscise?

STAIRS - Right, there was no evidence for strong parthenocarpic development in the material studied. However, as has been pointed out, it is possible that this is an artifact if the pollination bag creates an unfavorable environment.

TAFT - First I want to clear up one thing. The Florida and the Tennessee study are the same people. Donovan Forbes is currently employed by T.V.A., and he is also a graduate student of the University of Florida. One other thing, Jerry, on your pollination work, or actually the selfing work, it seems to me in talking with Forbes that when you can bag these racemes and then when you take them off to pollinate them, you can remove the flowers that have not come out if you want to. But if it is self-sterile, and sets no self seed, then you can put the bag right on without having to do this, and then the only seed that will develop are those that have been cross-pollinated. In other words, you have a whole series of flowers, and when you take your bag off to pollinate, apparently Don only does it once, and he pollinates those flowers that are then ready, acid then he puts the bag back on, assum ing that those that he did not pollinate won t ever develop seed anyway. So when he takes his bag off and collects his seed, the only seed that is developed is that which is crossed. Now I want to direct a question to Kitzmiller. Did you check the altitude of the seed collections from the south? If the Tennessee collection is one that I made, it came from 4000 ft. above sea level, whereas the Alabama collection probably came from only about 700 ft. above sea level.

KITZMILLER - Two of the Tennessee sources, including the one you collected near Knoxville, were significantly different from the Alabama sources in seed characters; however, the southern Tennessee source from Gile County showed similarities to the Alabama sources. Elevation may be an important factor; however, at present, our information is incomplete regarding elevations. We initiated a preliminary altitudinal transect study in the Morgantown area with elevations ranging from 800 to 2600 feet, taking 4 trees at every 300 foot intervals. Seed weights of trees from different elevations were not significantly different.

KLEIN - Do you happen to know whether that tree from Vermont that was throwing the albinos was isolated or in a group of other trees?

KITZMILLER - The mother tree was from Wisconsin and was graving in a forest stand, but it may or may not have been isolated.

ECKERT - Is the seed weight increased due to an increase in seedcoat thickness or is this an increase in the amount of endosperm?

KITZMILLER - Endosperm size or weight may contribute more to the differences in seed weight than endocarp thickness; however, we did not investigate the relationship between seed weight and endosperm size. Endocarp thickness and seed weight were significantly correlated in a positive direction.