VARIATION IN SYCAMORE SEED QUALITY

J.A. Mullins, Jr. and S.B. Land, Jr. [1/]

Abstract.--Type-M, "wet film" X-ray method provides better resolution of embryos in sycamore achenes than does "instant paper" X-ray process. "Wet film" radiographs allow designation of four embryo classes: full, partial, abnormal, and empty. Achene weight, percent germination, and rate of germination differ significantly between empties, abnormals, and the combined class of partials and fulls, but not between partials and fulls. Seven-day hypocotyl length does not vary significantly among embryo classes, but does among full achenes of different mother-tree seed lots. Significant phenotypic variation among families for achene weights, percent germination, and germination rate is also present.

Forest tree seed orchard managers must be concerned not only with seed yields, but also with seed quality. Quality here refers to the performance potential of the seed. Performance potential of every non-germinable seed is zero, but that of every germinable seed is not 100 percent (Delouche and Baskin, 1970). Seed vigor, as used in agricultural crop seed technology, is the measure of environmentally-caused differences in performance potential among seed lots of the same genetic variety. In this paper seed quality is used as an encompassing category of many measures of performance potential that are influenced by both environmental and genetic factors.

The achene, or true fruit of American sycamore (Platanus occidentalis L.), is often called the seed. But the seed is actually enclosed within the achene wall. The only non-destructive method of examining the seed embryo is by soft-tissue X-ray radiographs. Can such radiographs provide sufficient resolution to detect embryo differences, other than full and empty achenes, in sycamore seed lots? Furthermore, if such differences can be detected, do they reflect differences in performance potential, and what are measures of performance potential? Finally, is there significant phenotypic variation among sycamore seed lots in these measures of performance potential? Answers to these questions are being sought, and initial results of an ongoing study are provided in the present paper.

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[1] /Graduate student and Assistant Professor/Forester, Department of Forestry, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, Mississippi. Contribution Number 3098 of the Mississippi Agricultural and Forestry Experiment Station. The authors gratefully acknowledge the advice and assistance provided by Drs. J. A. Vozzo and F. T. Bonner of the U.S. Forest Service Forest Tree Seed Laboratory, Mississippi State University, in this study.

MATERIALS AND METHODS

Sixteen open-pollinated mother tree seed lots were used. Each mother tree represented a different seed source in the Midsouth (Figure 1). There were four seed sources on each of four latitudinal transects.

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Soft tissue X-ray radiographs, using the "instant paper" process (Belcher, 1974), were made of the sixteen seed lots, where each seed lot was represented by 1000 randomly-selected seed fixed in position on a self-adhesive paper label. Achenes were separated into classes, based on embryo appearance in the radiographs, by comparing achene positions in the radiographs with positions on the self-adhesive paper. After separation, each class for each mother tree lot was re-X-rayed using the M-type "wet film" process (Belcher, 1968). Numbers of misclassified embryos from the "instant paper" process were recorded and lots reseparated into classes based on "wet film" embryo appearance.

Weights to the nearest 0.1 milligram were taken on four 10-achene samples from each embryo class in each mother tree lot and expressed as grams per 1000 achenes. Two of these samples from each lot were surface sterilized by soaking in a one-percent hypochlorate solution (clorox) for 30 seconds and rinsing three times with distilled water. The two sterilized and two non-sterilized 10-achene samples from each lot were placed on moist blotter paper in a dark germination chamber set for twelve hours at 30° C and twelve hours at 20° C each day. Germination counts were taken at three, five, and seven days following placement of the achenes in the germinator. Hypocotyl lengths were measured to the closest millimeter on each surviving germinant on the seventh day. Germination index, which is a measure of rate of germination and thereby of performance potential, was calculated as follows:

$$\text{Germination Index} = [\%\ \text{germination at day 3}] + [(\%\ \text{germination at day 5})/2] + [(\%\ \text{germination at day 7})/3].$$

Ten seed of each embryo class (except empty seed) in each mother tree lot were planted in each of three replications in a nursery in May, 1975. Percent germination after eleven days in the nursery was measured, and additional measurements of germination and growth will be taken during the summer of 1975.

RESULTS AND DISCUSSION

X-ray Radiographs

The M-type "wet film" process provides additional resolution of sycamore achene embryos not given by the "instant paper" process. Full and empty achenes can be distinguished on "instant paper" radiographs, but there are a number of unclassified achenes having unclear embryo images. M-type, "wet process" film allows the detection of the following four embryo classes:

(a) Full -- Embryo image appears strong and fills the seed cavity.

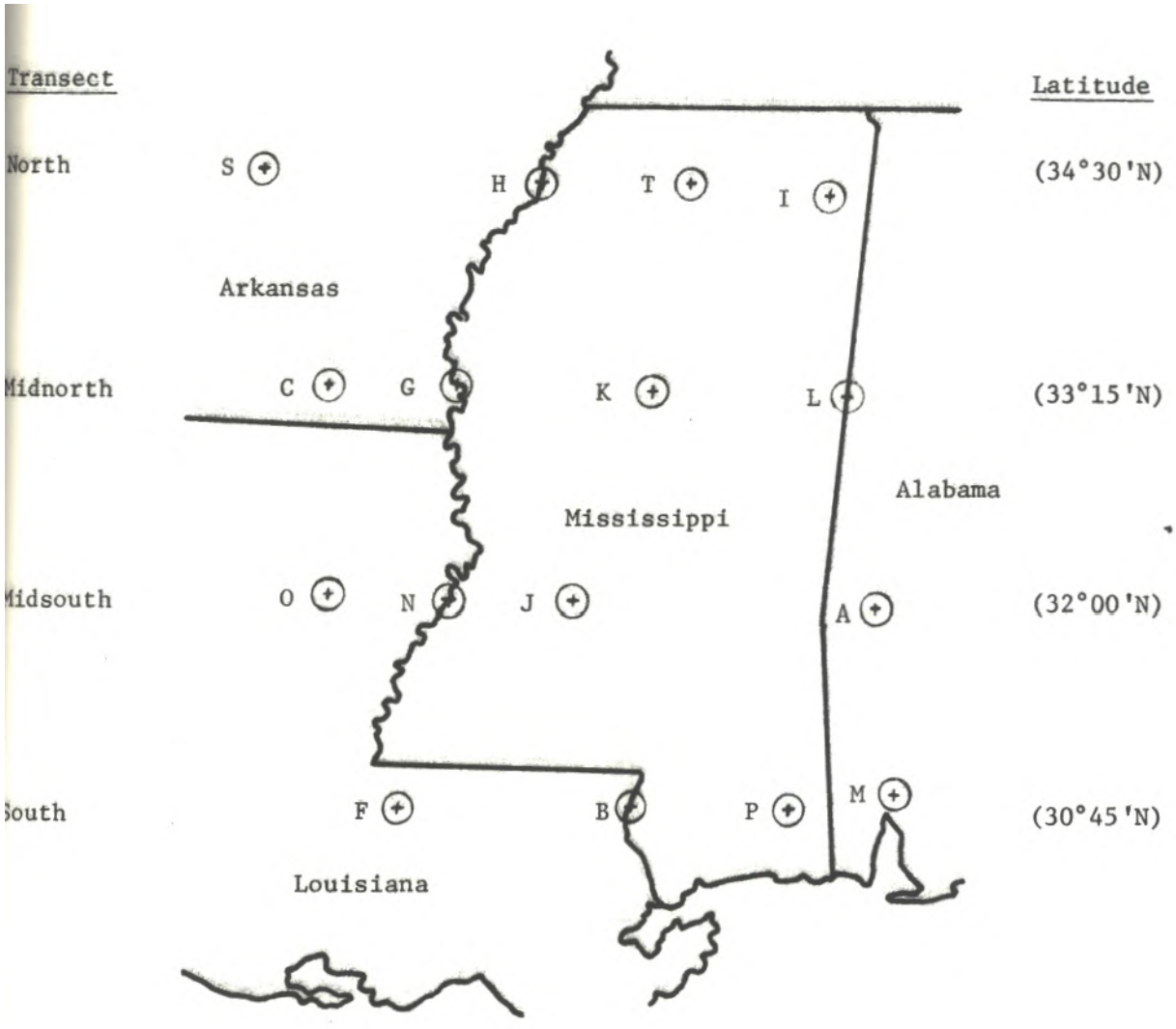

Figure 1.--Locations of mother trees used in study of sycamore seed quality.

(b) Partial -- Embryo image appears strong, but does not completely fill seed cavity.

(c) Abnormal -- Embryo image appears weak, incomplete, or shriveled.

(d) Empty -- No embryo image apparent.

Many of the full achenes from the "instant paper" process are determined to be partials by the "wet film" process, while unclassified achenes are primarily abnormals.

Variation in Seed Quality Among Embryo Classes

Highly significant variation in achene weight, percent germination, and germination index was detected among embryo classes (Table 1). But full and partial classes were never significantly different--the significant variation being among fulls and partials vs. abnormals vs. empties (Table 2).

Full and partial classes were heavier, had a higher percent germination, and germinated faster than abnormals. Abnormals, in turn, were correspondingly greater for these traits than empties.

Germination after eleven days in the nursery was similar to the above results in differences among the four embryo classes (Table 2). Full and partial classes may be combined in interpreting X-ray radiographs of seed lots for nursery production purposes, but abnormals and empties should be considered as non productive. The "instant-paper" X-ray process should be suitable for such interpretations, since the full class from this process contained most of the true full and partial achenes.

Hypocotyl length of surviving germinants after seven days in the germinator is not a suitable measure of differences among classes. What few seed germinated and survived from the empties and abnormals grew as tall as did those from fulls and partials.. The hidden difference among embryo classes is in the number that germinated and survived: 409 fulls, 362 partials, 125 abnormals, and seven empties of 640 seed per class.

Surface sterilization of achenes with dilute clorox resulted in both increased percent germination and faster germination. It also reduced the error component of variance (Table 1), thereby increasing precision in detecting differences among classes and among family seed lots. Such sterilization procedures are recommended for studies of phenotypic variation in sycamore seed quality.

Variation in Seed Quality Among Mother-Tree Seed Lots

Variation among mother-tree seed lots in percent germination and germination index was highly significant, but there was no apparent trend among latitudes of the sources (Tables 1 and 3). Interaction of families with embryo classes was also significant, because nearly all mother-tree-family

Table 1.--Analyses of variance for measures of sycamore seed quality from sixteen mother trees in the Midsouth

Source of Variation	d.f.	Achene Wt. M.S.	d.f.	M.S. 7-Day Germination (%) Steril.[a]	Unster.	Germination Index[b] Steril.	Unster.	d.f.	Hypocotyl Length (Ster. Full Embryos) M.S.
Family	15	17.32**	15	1193**	2739**	1435**	1221**	15	15.13**
Embryo Class	3	14.85**	2	29279**	18959**	26966**	4776**		
F x C	45	.25**	30	265**	244	368**	220**		
Error	192	.04	48	103	237	93	101	16	2.88

[a] Sterilized refers to seed that were surface sterilized with clorox.

[b] Germination index is an expression of rate of germination.

Table 2.--Sycamore seed quality means for four seed embryo classes and results of Duncan's New Multiple Range Tests[a/]

Embryo Class	Achene Wt. (gm./1000 achenes)	7-day Germination (%)		Germination Index[b/]		Hypocotyl Length at 7 days (mm.)	Nursery Germin. (%) at 11 days
		Sterilized	Non-sterilized	Sterilized	Non-sterilized		
Full	3.7 a	91 a	74 a	88 a	41 a	12.2	9
Partial	3.4 a	74 a	54 a	69 a	27 ab	12.9	9
Abnormal	3.1 b	33 b	14 b	32 b	13 bc	13.4	3
Empty	2.6 c	2	1	2	1	13.8	-

[a/] For a single seed quality measure, means not followed by the same letter are significantly different at the five percent probability level.

[b/] Germination index is a measure of rate of germination. Higher values reflect faster rates of germination.

Table 3.--Mother-tree family means and results of Duncan's New Multiple Range Tests[a] for sycamore seed germination and hypocotyl lengths

Mother-tree Seed Lot	7-Day Germination (%) (Surface Sterilized) Full	Partial	Abnormal	Germination Index[b] (Surface Sterilized) Full	Partial	Abnormal	Hypocotyl Length (mm.) (Sterilized, Full)
South Transect[c]:							
M	85a	65CD	25defg	80A	60cd	25DE	14.5ab
P	100a	45DE	5g	100A	35ef	5F	10.2c
B	95a	70BC	30cdef	95A	68bc	30DE	14.9ab
F	95a	85ABC	55ab	90A	85ab	55ABC	14.7ab
(Transect Mean)	(94)	(66)	(29)	(91)	(62)	(29)	(13.6)
Midsouth Transect:							
A	95a	80ABC	35bcde	84A	80abc	35CDE	9.2cd
J	90a	65CD	5g	88A	55de	5F	11.2bc
N	95a	95A	65a	95A	95a	65A	11.9cd
O	95a	70BC	35bcde	90A	65bcd	35CDE	9.8cd
(Transect Mean)	(94)	(78)	(35)	(89)	(74)	(35)	(10.5)
Midnorth Transect:							
L	50b	45DE	5g	50B	35ef	5F	11.4bc
K	85a	40E	20efg	83A	25f	15EF	6.2d
G	95a	90AB	50abc	93A	90a	43BCD	12.1bc
C	100a	75ABC	35bcde	98A	55de	30DE	9.9cd
(Transect Mean)	(83)	(63)	(28)	(81)	(51)	(23)	(9.9)
North Transect:							
I	85a	90AB	35bcde	78A	83ab	35CDE	15.2ab
T	100a	90AB	45abcd	100A	90a	45ABCD	16.5a
H	100a	95A	15efg	100A	95a	13EF	14.7ab
S	95a	90AB	60a	93A	90a	60AB	14.6ab
(Transect Mean)	(95)	(91)	(39)	(93)	(90)	(38)	(15.3)

[a] For a single seed quality measure, means not followed by the same letter are significantly different at the five-percent probability level.
[b] Germination index is a measure of rate of germination. Higher values reflect faster rates of germination.
[c] See transects in Figure 1.

variation was for partial and abnormal classes (Table 3). For definitive phenotypic studies of sycamore seed germination, family seed lots should be analyzed for each of the four embryo classes. Otherwise, differences among seed lots may be due to differences in percentages of each embryo type in each lot. "Wet-film" X-ray procedures will therefore be required in such studies of variation.

Germination index was not particularly better than percent germination for detection of family differences in the present study. Ninety-four percent of the germination occurred before the first measurement at three days, creating similar index and percent values. For germination index to be more definitive, measurements should also be taken on the first and second days.

Hypocotyl length of seedlings from the surface-sterilized, full achenes was highly significantly different among families (Table 1). Mean family lengths varied from 6.2 mm. to 16.5 mm. after seven days in the germinator. This measure of seed quality will be important in studies of phenotypic variation. Such studies will not require separation of embryo classes, since germinated seedlings from all four classes performed similarly for a family lot.

Mother-tree family variation in achene weight for each of the four embryo classes was highly significant. Mean full achene weights per 1000 achenes for families varied from 1.98 grams to 6.52 grams (Table 4). Empty achene weights, which were assumed to represent the weights of the achene fruit wall, ranged from 1.43 grams/1000 to 4.24 grams/1000. A mean "embryo" weight for each family was calculated as the difference between mean full weight and mean empty weight. Such "embryo" weights varied from 0.51 grams/1000 to 2.28 grams/I000 achenes, and 15 percent to 47 percent of full achene weight. Although the differences among these family means could not be tested for significance in the present study, they do indicate another measure that should be examined in studies of phenotypic variation.

SUMMARY AND CONCLUSIONS

The M-type "wet film" soft X-ray procedure provides better resolution of embryo images in sycamore achenes than does the "instant paper" process. Four embryo classes, based on embryo appearance, can be distinguished from "wet film" radiographs: full, partial, abnormal, and empty.

Full and partial embryo classes of achenes are not significantly different for weight, percent germination, or rate of germination. But abnormal and empty classes are successively and significantly lighter, poorer in total germination, and slower in germination than the fulls and partials. Surface sterilization of achenes with dilute clorox enhances germination, reduces error variation, and is recommended for use in seed quality phenotypic studies.

Phenotypic variation in seed quality is large for all measures tested. No trends associated with latitude of seed source are evident for the sixteen families used. Hypocotyl length of the seedlings after seven days in the germinator, weights of full achenes, and weights of empty achenes appear the most promising of the traits studied as measures of phenotypic variation in sycamore seed quality.

Table 4.--Mother-tree family means and results of Duncan's New Multiple Range Tests[a] for sycamore seed weights

Mother-Tree Seed Lot	Achene Weights (gms./1000 achenes) Full(F)	Empty(E)	Calculated Embryo Wt. = F - E (gms./1000 achenes) F - E	100%(F-E)/F
South Transect[b]:				
M	4.61d	2.86D	1.74	38
P	4.03e	3.02D	1.10	25
B	2.75gh	2.05E	.70	26
F	3.43f	2.92D	.51	15
(Transect Mean)	(3.71)	(2.71)	(1.01)	(26)
Midsouth Transect:				
A	4.03e	3.11D	.92	23
J	2.44h	1.43G	1.01	41
N	2.75gh	1.55EF	1.20	43
O	1.98i	1.46G	.52	26
(Transect Mean)	(2.80)	(1.89)	(0.91)	(33)
Midnorth Transect:				
L	4.74c	3.52B	1.22	26
K	2.97g	1.58EF	1.39	47
G	2.69gh	1.84EF	.85	32
C	6.52a	4.24A	2.28	35
(Transect Mean)	(4.23)	(2.80)	(1.44)	(35)
North Transect:				
I	3.94e	2.84D	1.10	28
T	5.29b	3.51B	1.78	34
H	4.06e	3.17C	.89	22
S	2.92g	2.07E	.87	30
(Transect Mean)	(4.05)	(2.90)	(1.16)	(28)

[a] For a single trait, means not followed by the same letter are significantly different at the five-percent probability level.

[b] See transects in Figure 1.

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