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Testing for Seed Quality in Southern Oaks

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SUMMARY

Expressions of germination rate, such as peak value (PV) or mean germination time (MGT), provide good estimates of acorn quality, but test completion requires a minimum of 3 weeks. For more rapid estimates, tetrazolium staining is recommended. Some seed test results were significantly correlated with nursery germination of cherrybark and water oaks, but not with seedling growth.

Additional keywords: Acorns, germination, nursery performance, *Quercus*.

INTRODUCTION

Total production of bareroot seedlings of southern oaks is not large, although most nurseries do grow some oak seedlings. For the 1982-83 planting season, nine states advertised seedlings from 1 to 3 species of oak (Monaghan 1982). While several industrial nurseries also grow oak seedlings, their supply is destined to reforest their own lands.

Along with this limited resource of seedlings, nurserymen face the fact that collection and care of acorns are more difficult than for most tree seeds, so reliable data on acorn quality becomes important in obtaining seedlings efficiently. Some factors that contribute to this difficulty are:

(1) Bumper crops are erratic and greatly variable in quality among seed lots.
(2) Premature collection of acorns is very common when collecting from logging slash; full maturity, necessary for normal germination, may not be apparent to the eye.
(3) Post-collection care is difficult. Viability can be lost quickly from excessive drying, freezing, or heating.
(4) Acorns must be stored above freezing at high moisture contents.

These conditions also favor insects, disease, and early sprouting.

OPTIONS FOR TESTING

Several options exist for evaluating acorn quality:

A. Standard laboratory germination test. Germination tests can be run using the "cut and peel" method which is very similar to techniques used for official seed tests (Association of Official Seed Analysts 1978). The procedure is simple:

1. Cut acorns in half (pruning shears are good for this) and discard the half with the cup scar.
2. Peel the pericarp from the remaining half and place it on moist blotter, Kimpak[1], or similar material, cut side down.
3. Incubate at alternating temperatures of 20° (16 hours)/30°C (8 hours, with light). Keep test surface moist.
4. Count germination at desired intervals for up to 28 days. (Tests may be terminated early if germination is complete.) An acorn is scored as germinated if both radicle and shoot exhibit normal growth and morphology.

B. Rapid viability tests. Because of time restrictions mentioned earlier, seed managers may prefer one of three rapid viability estimates:

1. Cutting test. Cross-section cuts will disclose damage due to insects, disease, and desiccation, but

[1]Mention of trade names is for information only and does not imply endorsement or recommendation by the U.S. Department of Agriculture over others not mentioned.

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they may not always disclose loss of viability from overheating or freezing.

2. Radiography. X-rays can provide a very quick, non-destructive test for damage or incomplete embryo development. Sample exposure and radiograph interpretation guides are available (Belcher and Vozzo 1979).
3. Tetrazolium staining (TZ test). Tetrazolium chloride is widely accepted as a vital stain in seed science. The following procedure has been moderately successful for acorns:
   (a) Use two samples of 50 acorns each.
   (b) Cut acorns in half with pruning shears. Remove pericarp from the half containing the embryonic axis, and discard the other half. Count and discard any rotten acorns.
   (c) Place acorn halves in a deep dish, micropyle end down, and add TZ solution until the halves are almost covered. Solution should be made from 2,3,5-triphenyl tetrazolium chloride and should have a pH of 7.0.
   (d) Cover dishes and incubate in the dark for 24 hours at 21° to 25°. Break cotyledons apart to study stain in each acorn. A good stain is bright pink to red. Very dark red (as in blood) indicates tissue damage.
   (e) Score stain in 5 classes:
      I. Cotyledon and embryonic axis both unstained.
      II. Axis stained, cotyledon unstained.
      III. Cotyledon stained, axis unstained.
      IV. Axis and cotyledon both unstained.
      V. Rotten or insect damage beyond recovery.

   Class I is best, of course, but many acorns in Class II will also germinate. Class III acorns are very weak, and they normally will not produce plantable seedlings in nurseries. Some may germinate in greenhouses. Classes IV and V will not germinate, but inclusion of the counts can show the reasons for poor quality.

C. Vigor tests. Germination or viability percentages seldom give a completely accurate picture of acorn quality. Several other tests may be used:

1. Germination rate. Speed of germination may be expressed as mean germination time (MGT), or peak value (PV) where PV = maximum value of cumulative percentage divided by days of test (Czabator 1962).
2. Leachate conductivity. Immersion in distilled water for various periods, followed by some measure of solute leakage into the water, has been used successfully for many seeds. Electrical conductivity of the leachate is the most popular method, and good correlations between leachate conductivity and seed vigor in water oak acorns have recently been reported (Agmato 1982).

EXPERIMENTAL RESULTS

Various experiments have been carried out on measurement of acorn vigor at the Forestry Sciences Laboratory in Starkville, Mississippi, since 1972. Some results have been published previously for cherrybark oak (Bonner 1974). More recent results are herein summarized:

A. 1978 Water Oak Test Methods

Five lots of water oak collected from 1975 to 1978 were randomly sampled for the following tests:

1. Standard laboratory germination test.
2. TZ test.
3. Nursery growth tests which measured:
   a. bed germination.
   b. bed survival at end of growing season.
   c. seedling dry weight at end of growing season.

All lots had been cleaned and upgraded by water flotation at collection. Prior to testing they had been stored moist at 2°C, according to current recommendations (Bonner 1973).

The germination tests employed 4 samples of 50 unstratified acorns each. Germination was counted 3 times per week. TZ tests were carried out on 4 samples of 50 acorns each, except for lot 77-17, which had only 2 samples available. Nursery growth plots were four completely randomized plantings of 50 acorns each, which had received 36 days of stratification at 3°C.

Results—All germination and TZ tests clearly showed that lot 75-5 exhibited poorest quality and lot 77-17 was the best (table 1). Values for the other 3 lots were intermediate with few significant differences. TZ results for lot 77-17 were much higher (95%) than those of the other lots, and TZ and germination capacity were about equal.

Nursery germination and survival were similar to the laboratory germination test results, although lot 77-17 did slip in final nursery survival (49.2%—third best). Mean seedling dry weights were highest for 77-17 and 75-5 (the lot of lowest quality). The high mean weights for lot 75-5 are thought to be related to low bed density resulting from poor germination. No plots were thinned to a uniform density, a condition which was corrected in the next nursery test.

These results suggested that laboratory germination capacity, germination rate (PV), and TZ test results were all correlated with nursery germination and growth, but the number of lots precluded a definitive test. The big differences between germination and TZ results for some lots seemed inconclusive.

B. 1982 Test Methods

Multiple lots of white oak (12), cherrybark oak (15), and water oak (15) were used to retest the relationship of seed vigor estimates to seedling performance in nursery beds. The following tests were run:

1. Standard laboratory germination test.
2. TZ test.
3. Nursery growth tests which measured:

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Table 1.—*Germination and seedling growth in 5 lots of water oak, 1978 test*

Seed Lot*	PV	Germination capacity	TZ stain Class I	Nursery germ.	Nursery survival	Seedling dry weight
		pct.				g
75-5	4.0 a	58.2 a**	11.0 a	32.8 a	30.8 a	2.1 a
77-6	5.8 b	84.0 b	44.0 b	46.0 ab	42.0 ab	1.3 b
78-7	5.8 b	90.8 b	61.0 c	56.8 bc	51.6 bc	1.7 ab
76-10	6.6 bc	90.8 b	51.0 b	60.8 bc	60.0 c	1.3 b
77-17	7.0 c	98.0 c	95.0 d	65.6 c	49.2 bc	2.0 a

*The first two numbers in the lot designation refer to year of collection.
**Lot means followed by the same letter did not differ significantly at the 5% level, based on Duncan's new Multiple Range test.

Table 2.—*Laboratory germination and TZ stain data for all 3 species, 1982 test. Rate parameters (PV and MGT) were not available for white oak*

	White oak		Cherrybark oak				Water oak			
Lot no.	TZ stain	Germination capacity	TZ stain	Germination capacity	PV	MGT	TZ stain	Germination capacity	PV	MGT
	pct.					days	pct.			days
1	84	96.5	8	9	1.4	7.4	80	71.0	4.3	13.2
2	83	90.5	89	83	11.2	5.9	64	94.0	10.9	7.9
3	71	81.5	94	86	10.4	5.9	45	52.0	4.7	9.8
4	90	96.5	86	93	14.4	4.4	85	83.5	4.3	15.3
5	82	84.0	86	93	12.7	5.0	71	71.0	6.1	8.6
6	88	80.0	48	37	4.0	6.9	65	87.5	5.1	13.7
7	78	70.0	97	92	10.0	7.1	82	93.5	11.4	4.8
8	96	83.5	86	77	5.9	12.4	70	89.0	10.2	8.4
9	75	62.0	88	77	8.2	6.9	89	87.5	5.0	13.5
10	84	84.5	90	82	8.2	7.1	86	83.5	8.4	8.7
11	77	67.0	80	84	12.0	5.3	83	87.0	9.0	8.3
12	78	67.0	....	....	....	....	75	92.5	6.1	12.8
13	89	89.0	....	....	....	....	78	94.5	6.6	11.3
14	86	75.0	....	....	....	....	76	81.0	10.1	7.8
15	82	65.0	....	....	....	....	66	92.5	7.6	10.0

a. weekly emergence.
b. number of plantable seedling produced.
c. mean seedling fresh weight, top length, stem diameter, and volume (D^2H).

Upgrading, storage, and sampling paralleled other tests. Germination was tested with 4 samples of 50 acorns each, TZ tests with 2 samples of 50 each, and nursery growth with 4 samples of 50 each.

White oak acorns were planted in February without stratification. Almost all acorns had emerging radicles visible at planting. Cherrybark and water oak acorns were stratified for 32 days at 2°, then planted on March 24. Many acorns had also started germinating by planting time. In late May all plots were thinned to a density of 4 to 6 seedlings per square foot. Beds were not fertilized and only moderately irrigated.

Correlation coefficients were calculated between all seed vigor estimates and all seedling performance parameters.

Results—For white oak, laboratory germination capacities ranged from 62.0 to 96.5 percent, while the TZ percent (Class I only) ranged from 75 to 96 percent (table 2). There was a significant positive correlation between the two ($r = 0.671$).

Seedling growth data for white oak indicated two things (table 3). First, once thinned to a good density, few performance differences showed between seed lots. Secondly, there were no significant correlations of seed vigor estimates with seedling growth. For these 15 lots of white oak, seedling performance could not be predicted by measuring seed quality.

Cherrybark oak test data showed a greater range of seed quality than that for white oak (table 2). Germination capacity values were very close to the TZ results ($r = 0.965$). Expressions of germination rate (PV and MGT) varied widely between lots, the variation suggesting that the rate parameters are more sensitive to vigor differences than are germination capacity percentages. This same relationship can be shown with seeds of many species.

Nursery growth results for cherrybark oak showed high positive correlation ($r = 0.983$) between TZ and nursery germination (table 4). Other significant, but lower, correlations with nursery germination were obtained with PV and

germination capacity. These two expressions and TZ were also significantly correlated with the number of plantable seedlings. However, none of the seedling growth parameters were correlated with estimates of acorn vigor.

Water oak germination and TZ results were similar to those in the 1978 test, because TZ was not significantly correlated with germination capacity (table 2). The only significant correlations between seed vigor estimates and nursery performance occurred in measures of germination rate: PV and MGT (table 5). MGT was significantly correlated with seedling size and weight, the only such significant correlation of the entire test. TZ and germination capacity were not correlated with anything.

DISCUSSION

Species differences cause some problems in TZ staining of acorns. Water oak cotyledons fit together very tightly, thus restricting access of the TZ solution to the embryonic axis. This species also has a tough testa next to the cotyledons, which restricts entrance of the solution. Similar problems have occurred in other tests with willow oak and Nuttall oak acorns. Scraping the testa away from the embryonic axis and removing more than one-half of the cotyledons might improve the condition. The more cotyledon removed, the more likely that the remaining pieces will separate when imbibed.

Cherrybark oak acorns do not present the problems described above. Their cotyledons readily separate when

Table 3.—*White oak seedling performance, 1982 test*

Lot no.	Nursery emergence	Nursery germination	Plantable* seedlings	Fresh** weight	Top length	Stem diameter	D^2H
	wks.	*pct.*	*no.*	*g*	*cm*	*mm*	*cm^3*
1	8.0	46.0	11	51.1	46	8.8	38.6
2	7.8	89.5	16	65.0	58	9.2	56.6
3	7.7	69.5	17	71.2	60	9.6	59.4
4	8.3	46.5	12	57.0	48	8.4	36.1
5	7.5	82.0	14	70.2	55	9.6	54.6
6	8.0	66.0	10	57.4	39	8.2	28.4
7	8.3	43.0	9	35.0	40	6.8	21.0
8	8.2	34.0	7	46.6	39	8.5	30.0
9	8.8	49.5	15	66.5	61	9.6	61.3
10	7.9	65.5	16	56.3	56	9.0	48.4
11	8.1	60.5	13	57.8	51	8.8	42.1
12	7.7	44.5	15	65.4	55	9.5	52.8
13	7.8	56.5	14	54.4	56	8.9	48.5
14	8.3	51.5	14	75.7	66	9.8	71.0
15	8.7	26.0	8	53.1	43	8.5	36.1

*Minimum top length of 30 cm.
**All roots pruned to a length of 15 cm before weighing.

Table 4.—*Cherrybark oak seedling performance, 1982 test*

Lot no.	Nursery emergence	Nursery germination	Plantable* seedlings	Fresh** weight	Top length	Stem diameter	D^2H
	wks.	*pct.*	*no.*	*g*	*cm*	*mm*	*cm^3*
1	8.0	2.0	2	28.4	52	7.5	32.4
2	6.7	75.0	19	52.4	77	9.0	64.2
3	7.3	73.5	18	22.9	49	6.2	19.8
4	7.6	81.5	17	26.0	48	6.2	18.8
6	6.2	79.0	20	45.3	72	8.2	51.3
7	7.3	44.5	18	51.1	68	8.8	56.0
8	7.1	83.0	20	46.9	70	8.8	55.3
9	7.2	76.5	20	50.5	75	8.9	61.3
10	6.7	75.0	20	49.4	76	8.7	60.6
11	6.1	84.0	20	49.9	78	8.6	59.8
12	5.7	71.5	20	52.9	79	8.7	63.2

*Minimum top length of 30 cm.
**All roots pruned to a length of 15 cm before weighing.

Table 5.—*Water oak seedling performance, 1982 test*

Lot no.	Nursery emergence	Nursery germination	Plantable* seedlings	Fresh** weight	Top length	Stem diameter	D^2H
	wks.	*pct.*	*no.*	*g*	*cm*	*mm*	cm^3
1	9.8	35.5	16	39.1	65	7.6	40.8
2	8.1	67.0	20	50.0	71	8.9	60.0
3	7.6	45.5	18	52.8	74	8.4	57.8
4	10.2	22.5	12	26.7	52	6.6	25.1
5	7.8	59.5	20	63.4	76	9.6	74.8
6	9.7	44.0	18	27.0	57	6.6	27.4
7	8.4	62.0	19	42.9	67	8.3	48.6
8	8.2	75.0	19	37.2	60	7.5	36.7
9	10.1	33.0	15	32.2	59	7.4	36.4
10	9.0	60.5	19	56.8	74	8.9	61.9
11	7.2	64.0	20	62.0	81	10.2	90.6
12	10.0	46.5	17	37.7	60	8.0	40.2
13	8.7	58.5	18	53.0	78	9.0	67.2
14	8.8	69.0	19	41.6	66	7.8	42.8
15	9.2	60.5	18	57.2	73	9.0	64.2

*Minimum top length of 30 cm.
**All roots pruned to a length of 15 cm before weighing.

imbibed, and the testa on cherrybark acorns is thinner. Other red oak species that are more like cherrybark than water oak are Shumard and scarlet oaks.

Cotyledon color also influences stain interpretation. Healthy acorns with high lipid contents (water, willow, cherrybark) are orange, and the stain will show reddish-orange. Low-fat acorns (Shumard, all white oaks) have white to pale yellow cotyledons; good stains in these cases are typically pink to red.

Stain interpretation was also difficult on white oak acorns, because the radicles were, in most cases already protruding through the pericarp when the tests were run. Rapid germination of this species is the rule, not the exception. When white oak acorns are cut, the cotyledons frequently break apart and damage the axis.

In general, correlations between laboratory seed measurements and seedling growth in the nursery were poor. Only MGT for water oak gave significant correlations with seedling growth. The strongest relationships were between nursery germination and the number of plantable seedlings. Once seedlings were established and bed densities equalized, seed quality had little effect on seedling size. Had bed densities not been adjusted, though, the best lots would have been too dense in these tests, and seedling growth would have suffered.

It is also apparent that seed measurements that express rate of germination were the best indicators of nursery performance. This conclusion, consistent with the bulk of seed research results, was reported by our laboratory previously for cherrybark oak (Bonner 1974).

The major question remaining is: "Why weren't the seed measurements correlated with seedling performance?" Definitive answers are not apparent, but the nature of the oak seed itself is influential. Acorns have an abundant food supply within themselves. Much damage can occur and can be reflected in TZ tests, yet the acorn can still germinate and produce a normal seedling. This has been observed many times in our laboratory and nursery, and it could explain the germination capacities' being much higher than the TZ values for water oak in 1978 (table 1).

Acorns are recalcitrant seeds with short life spans. Once seed quality begins to decline, it declines very rapidly. The time course of acorn deterioration is so rapid that seed lots are either good or very bad within three years of collection.

CONCLUSIONS AND RECOMMENDATIONS

1. Several seed vigor tests were significantly correlated with nursery germination of cherrybark and water oaks, but not for white oak. Tetrazolium (TZ) staining was best for cherrybark oak; peak value (PV) was also good. Germination rate expressions (PV and MGT) were best for water oak.

2. Seed vigor tests could not predict oak seedling performance, as measured by seedling growth parameters. Only mean germination time (MGT) for water oak was significantly correlated with seedling growth, and these coefficients were low (0.59 to 0.54).

3. TZ results were significantly correlated with germination capacity (lab test) for white oak and cherrybark oak, but not for water oak. Cherrybark oak is well-adapted to the TZ test, but water and white oaks present minor problems.

4. In spite of mixed results just discussed, seed quality tests are still definitely recommended for seed managers and nurserymen who must make management decisions or calculate sowing rates for seed lots of southern oaks. If

immediate decisions (those being made within 7 days) are required, TZ tests should be made. The procedures described in this paper are easily learned. If more time is available (3 weeks or more), germination tests that allow calculation of rate measures (PV or MGT) should be used. As to "cut and peel" preparation, which is similar to that for the TZ test, that method's germination data should be available in 14 to 21 days for most species.

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