### MEASURING SOUTHERN PINE SEED QUALITY WITH A CONDUCTIVITY METER--DOES IT WORK?

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Abstract.--Preliminary tests of an ASA-610 Automatic Seed Analyzer from Agro Sciences, Inc. with five species of southern pines indicate that valid estimates of seed quality are possible from leachate conductivity measurements. Several factors which influence the results are discussed. Studies still in progress are expected to bring measurement errors to within acceptable limits.

Additional keywords: Germination, seed tests, loblolly pine, slash pine

Of all the methods proposed to measure seed quality in recent years, one has become the basis for commercial development of equipment to do the job. The machine used in this process is the ASA-610 Automatic Seed Analyzer, developed and marketed by Agro Sciences, Inc. of Ann Arbor, Michigan.2/ This machine measures the electrical conductivity of water in which a seed has been soaked. The amount of current passing through the soak solution is influenced by the amount of solutes leaching from the seed, which, in turn, is theoretically related to the vigor of that seed as a function of membrane integrity. This is a non-destructive test, and the seeds can be subsequently germinated or dried for storage.

It is assumed that as a seed deteriorates, its membranes break down and allow the leaching of internal substances. Murphy and Noland (1982) found that heat-killed embryos of sugar pine had higher rates of solute leakage than did viable ones, and Pitel (1982) demonstrated that increasing periods of accelerated aging of jack pine seeds resulted in increased conductivity of the soak water. Hocking and Etter (1969) reported a close correlation between germination of white spruce and the sugar concentration in seed leachate. The relationship of leached solutes and field emergence was first demonstrated for peas in 1968 (Matthews and Whitbread 1968). Later research has supported the principle, and the International Seed Testing Association has included an electrical conductivity test in its Handbook of Vigor Test Methods (ISTA 1981).

The first machine marketed by Agro Sciences to measure seed quality used a forcep-type electrode system which measured current passing through a single seed. The ASA-610 now on the market has a multiple-electrode plate which fits onto a tray with 100 uniform compartments for soaking the seed sample (usually one seed per compartment). When the plate is placed on the tray, the

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<sup>2/</sup> Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U. S. Forest Service and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

electrode pairs are immersed in the soak solutions. The ASA-610 then measures the individual current levels in 100 seed compartments simultaneously.

This new model has shown great promise with agricultural seeds (McDonald and Wilson 1979). The potential value of such a method for quick estimates of the seed quality is enormous. An ASA-610 was acquired for the Forestry Sciences Laboratory in 1981, and tests were begun on southern pine seeds.

## MATERIALS AND METHODS

The basic approach to evaluation of the ASA-610 was to draw samples from a wide variety of seed lots, take conductivity readings, and then germinate these same seeds in the laboratory. Seed lots over a wide range of ages (collected 1967 to 1981) from throughout much of the native range of the species were used. Tests were carried out on 25 lots of loblolly, 25 of slash, 6 of shortleaf, 7 of longleaf, and 4 of eastern white pine.

Several factors influence the current readings in the solutions: (1) amount of soaking time, (2) temperature of the solution, (3) water level in the measurement cells (seed:water ratio), and (4) initial seed moisture content. After extensive preliminary testing, the following conditions were selected for standardization of measurement technique:

- (1) soaking time 48 hours
- (2) solution temperature 25+1°C (laboratory temperature)
- (3) water level cells uniformly full (4 ml deionized water)
- (4) initial seed moisture content 10 to 12% or lower (only dry seed lots from storage were used).

There were two approaches to evaluation:

(A) Individual Seed Response - Conductivity measurements for individual seeds were related to the number of days required for germination of those seeds. Conductivity measurements were taken on two samples per lot. These seeds were then germinated in cabinet germinators set for the standard 20°/30°C alternating regime (AOSA 1981). Leachate conductivities were recorded on tape, and seed identities were maintained during the germination tests. Simple correlation coefficients were calculated.

(B) Entire Sample Response - As in germination testing, response of a suitable sample is more likely to reflect the condition of the population as a whole than are measurements on individual seeds. One approach suggested by the manufacturer is to set a threshold value of cell conductivity. These values are called "partition values," and they theoretically separate live and dead seeds. Partition values usually vary among species and must be chosen empirically. By plotting the frequency distribution of individual seed conductivities and comparing these data to germination results, trial partition values, were chosen. In subsequent tests, the meter was set on these partition values, and the readout gave the number of seeds whose conductivity was lower than the partition value.

In addition to partition value estimates, mean conductivity of the 100-seed samples and the standard deviation about the mean (a measure of uniformity) were related to the germination response of the samples from each lot by linear regression. Germination tests were done in accordance with the rules of the Association of Official Seed Analysts, which establishes standard seed testing procedures (AOSA 1981).

3.

1

#### RESULTS

(A) Individual Seed Response - This approach proved fruitless. The scatter of a typical sample (figure 1) shows the poor relationship between leachate conductivity and speed of germination. All correlation coefficients were extremely low and non-significant at the 5 percent level.



Figure 1.--Relationship of leachate conductivity and the number of days until germination for individual seeds of slash pine.

(B) Sample Response - For loblolly and slash pines, conductivity readings (mean of duplicate samples of 100) were significantly correlated with laboratory germination. The relationship was much stronger in slash, where an r value of -0.8939 was obtained for laboratory germination and mean conductivity (figure 2). This same comparison gave an r value of -0.6502 for loblolly (figure 3).

For shortleaf, longleaf, and eastern white pines, too few lots were available to make these analyses, but a summary of the data suggests that a similar relationship may exist (table 1).

Graphic analysis of the data was used to pick the most likely partition values for loblolly and slash pines. Rearrangement of the germination data in descending order facilitates this comparison (table 2). It can be quickly

99



Figure 2.--Relationship of laboratory germination to mean leachate conductivity for 25 lots of slash pine. Each value is the mean of two replicates.



Figure 3.--Relationship of laboratory germination to mean leachate conductivity for 25 lots of loblolly pine. Each value is the mean of two replications.

	Laboratory germination	Mean conductivity	Standard deviation of conductivity			
	percent	microamp	microamp			
Shortleaf	70	43.1	22.1			
	68	44.4	18.4			
	63	48.0	20.8			
	44	43.2	13.3			
	4	43.2	9.4			
	2	43.2	7.8			
Longleaf	84	101.4	95.6			
	68	93.4	95.6			
	68	103.5	99.3			
	58	153.0	134.8			
	38	156.5	127.6			
	18	220.0	160.2			
	10	355.0	203.6			
Eastern white	44	59.6	45.5			
	34	72.0	49.6			
	18	56.6	34.0			
	10	132.8	70.2			

# Table 1.Relationship of laboratory germination to conductivity of seedleachate for three pine species. Each value is the mean of tworeplications

seen that the ASA-610 did a better job of predicting slash than loblolly germination. Loblolly germination was overestimated, particularly in the poorer lots. The loblolly seeds were not stratified prior to germination testing, however, and this fact may have contributed to the overestimation.

To test the choice of partition values for these two species, the measurements were reported on 25 lots of each. Of the 25, 13 lots of loblolly and 20 lots of slash were repeaters from the first test. This time the loblolly received 28 days of stratification at 3°C between conductivity measurements and germination. Four samples of 100 seeds each from 50 lots (loblolly and slash) were also planted in randomly-placed rows in the Forestry Sciences Laboratory experimental nursery in a vigor evaluation test which purposely creates stressful conditions. Emergence was counted weekly for 6 weeks.

Results of this second test supported those of the first, and this time loblolly performance was much better correlated with the conductivity measurements. As before, however, germination of the best loblolly lots was slightly underestimated, while that of the poorer lots was overestimated (table 3). The same condition existed with the slash lots, but to a lesser degree (table 4). Simple correlation coefficients between nursery emergence at 6 weeks and

Loblo	11y	Slash							
Laboratory	Predicted	Laboratory	Predicted						
germination	by ASA-610 <u>a</u> /	germination	by ASA-610 <u>b</u>						
	perc	ent	**						
89	96	91	90						
86	97	90	92						
75	94	88	92						
74	78	88	98						
72	61	87	71						
68	84	86	90						
68	68	85	94						
66	73	84	97						
59	81	83	86						
56	74	80	74						
56	58	79	88						
54	73	76	88						
54	69	76	74						
51	58	74	89						
48	92	71	82						
44	77	70	61						
41	82	64	74						
38	66	64	49						
32	84	62	66						
18	77	57	24						
16	96	56	72						
16	67	56	38						
2	18	50	52						
1	12	20	22						
0.5	2	14	21						
Mean									
47	69	70	71						

Table	2.	Lab	orator	ry ge:	germination and			rminat:	ion pre	dicted	by the ASA-610 fo			
		25	lots	each	of	loblolly	and	slash	pines.	Each	valu	ie is	the	mean
		of	two 1	repli	cati	lons								

 $\frac{a}{b}$  Partition value = 70.

 $\overline{\mathbf{b}}$ / Partition value = 80.

mean conductivity of seed leachates was -0.6390 for loblolly and -0.6576 for slash (both significant at the 5 percent level). Correlation coefficients between laboratory germination and mean conductivity were also significant: -0.8197 for loblolly and -0.8497 for slash. The higher coefficients for laboratory germination were not surprising, as additional environmental factors which can inhibit germination abound in nursery beds.

	Loblolly					
Laboratory germination	Predicted by ASA-610 <u>a</u> /	Nursery emergence @ 6 weeks				
	percent					
96	86	57				
92	85	60				
88	68	60				
88	84	56				
88	78	57				
88	40	49				
85	62	46				
84	86	43				
80	88	59				
80	88	38				
79	64	48				
78	82	47				
75	87	56				
74	73	28				
74	62	45				
68	76	42				
67	72	46				
66	42	45				
63	44	32				
58	68	32				
56	52	13				
52	73	27				
49	46	22				
39	55	18				
1	12	1				

# Table 3.--Laboratory germination, germination predicted by the ASA-610, and nursery emergence at 6 weeks for 25 lots of loblolly pine.

a/ Partition value = 70.

At this stage of the work, the following conclusions seem reasonable:

- The principle of the method is biologically valid, and significant correlations between seed quality and leachate conductivity can be shown.
- (2) With the methods of measurement used so far, variation is still large. Experiments are underway to solve this problem by studying such factors as:
  - (a) soaking time less than 48 hours may be sufficient.
  - (b) amount of seed with one seed per cell there seems to be better correlation as seed size increases (shortleaf < loblolly < white < slash < longleaf).</p>

Slash					
	Nursery				
Predicted (	emergence				
by ASA-610 <u>a</u> /	@ 6 weeks				
and the second sec	19 8 - 19 19 <b>20</b> 19 - 2 <b>20</b> 19 - 2 10 - 2				
percent					
94	41				
82	52				
94	39				
98	44				
89	38				
96	34				
80	46				
86	41				
82	43				
91	41				
84	32				
93	35				
84	57				
84	29				
84	37				
74	28				
80	39				
84	30				
88	34				
58	30				
90	38				
38	29				
68	27				
36	26				
42	16				
	Slash Predicted by ASA-610 <sup>a</sup> / 				

Table 4	4Laborator	y germina	germination,			on	predicted		l by	the	he ASA-610,	
	nursery	emergence	at 6	weeks	for	25	lots	of	slas	h pi	ine	

a/ Partition value = 80.

- (c) cleanliness of seed dirty seed lots give more variation a standard preliminary wash may help.
- (d) agitation during leaching this could easily be standardized and perhaps cut down on test time.
- (e) temperature during leaching higher temperatures might speed the measurement time.
- (f) pretreatments stratification or chemical treatments should be studied.
- (3) Leachate conductivity measurements will probably never match the precision of germination tests, but there is a great deal of interest in a reliable measure of seed quality that can give results within 24 hours without the subjectivity of X-ray or tetrazolium tests.

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