Leachate Conductivity: A Rapid Nondestructive Test for Pine Seed Quality

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Simple measurements of leachate conductivity from 100-seed samples can be used to roughly estimate viability or to assign seed quality classes of shortleaf (Pinus echinata Mill.), sand (P. clausa [Chapm ex Engelm.] Vasey ex Sarg.), Virginia (P. virginiana Mill.), jack (P. banksiana Lamb.), and Scotch pines (P. sylvestris L.). Results with red pine (P. resinosa Ait.) were unsatisfactory. This nondestructive test can provide 24-hour estimates of seed quality when germination tests are not possible. Tree Planters' Notes 42(2):41-44; 1991.

The need for a rapid yet simple method of estimating seed viability is readily apparent to all who grow tree seedlings. Laboratory germination tests are always preferable, of course, but time constraints frequently prevent their use.

A simple procedure based on the electrical conductivity of seed leachates has recently been developed for the southern pines (3). This test is based on the principle that seeds leach numerous substances when soaked in water and that because of membrane deterioration the amounts leached will increase as the seeds deteriorate. Although several chemical constituents can also be determined, the electrical conductivity of the leachate can be measured more quickly and easily.

In earlier work (3), the poorest results were obtained with shortleaf pine (*Pinus echinata* Mill.), perhaps because of its small size. Small seeds have less embryo tissue mass for potential loss of ions than larger seeds. This paper reports new results for shortleaf, as well as results with five other pines with small seeds (more than 40,000/Ib): sand pine (*P. clausa* [Chapm. ex Engelm.] Vasey ex Sarg.), Virginia pine (*P. virginiana* Mill.) jack pine (*P. banksiana* Lamb.), Scotch pine (*P. sylvestris* L.), and red pine (*P. resinosa* Ait.). Comparisons of germination and leachate conductivity for jack pine have also been reported by Pitel (5).

The USDA Forest Service National Tree Seed Laboratory, Macon, GA, supplied many of the samples used in this study.

Materials and Methods

The seedlots used in these tests were of various ages and from many different locations. All had been cleaned and stored at 2 °C at the Forestry Sciences Laboratory in Starkville, MS. Test procedures were similar to those used previously (3):

1. Two samples of 100 seeds each were counted out. All attached wing fragments were removed.
2. Samples were weighed to 2 decimal places.
3. Samples were rinsed in running tapwater for 15 seconds in a strainer, then placed in beakers with a measured amount of deionized water.
4. The samples were stirred with a Teflon spatula to break surface tension, and the beakers were covered with petri dish halves or watch glasses.
5. All beakers were placed in a dark incubator set for 20 ± 1 °C. Two blanks (beakers of deionized water without seeds) were also placed in the incubator.
6. After 24 hours, the contents of each beaker were poured through a mesh strainer to remove the seeds. The leachate was poured back into the original beaker for measurement of electrical conductivity with a YSI Model 32 conductivity meter manufactured by Yellow Springs Instruments.
7. The mean of the two blanks was subtracted from the readings and divided by the sample weight to obtain conductivity as microsiemens per gram (µS/g).

Three water volumes (100, 50, and 25 ml) were tested with 20 samples each of 100 seeds for each species-water combination (table 1). The objective was to see if lower water-seed ratios would improve accuracy with these small seeds. Conductivity tests with peas and soybeans typically use 25 seeds to 75 ml of water (2). The water volume that yielded the best regression for each species (highest R^2) (table 1) based upon the 20

Table 1—*Regression model* fits (R^2) for 25, 50, and 100 ml of water to a sample of 100 seeds*

Water volume (ml)	R^2
Sand pine	
100	.481
50	.143
25	.068
Shortleaf pine	
100	.379
50	.283
25	.343
Virginia pine	
100	.609
50	.337
25	.491
Jack pine	
100	.224
50	.367
25	.469
Red pine	
100	.178
50	.165
25	.339
Scotch pine	
100	.673
50	.382
25	.433

*Percentage germination = a + b (μS/g).

samples of 100 seeds was then used exclusively for subsequent studies of that species.

Seed samples were then germinated in the laboratory according to test prescriptions of the Association of Official Seed Analysts (1). Germination percentages were regressed on corresponding conductivity values for each seedlot within species (table 2). Several transformations of data were used to obtain the best fitting linear models based on R^2 values and standard errors of estimate for the regressions for each species using the water volume that yielded the best model in table 1.

Results and Discussion

Based on R^2 values for the regressions, 100 ml of water was best for shortleaf, sand, Virginia, and Scotch pines, while the lowest volume of 25 ml was best for jack and red pines (table 1). There are no obvious reasons for these species differences; it may be simply seed coat thickness or permeability, or embryo composition of the individual species.

Transformation of germination and conductivity data produced moderately good fits for all species except red pine (table 2). Only 19 seedlots were tested; more data are apparently needed to develop a good model for this species. The shortleaf model gave a better fit than previously reported (3). The best fit was with Scotch pine (R^2 = .751) and the worst with red pine (R^2 = .339).

As in the previous work with southern pines (3), empty and dormant seeds were only a minor problem. Empty seeds have few electrolytes to leach, so they do not produce high leachate conductivity. Properly cleaned pine seedlots should have few or no empty seeds, but exceptions will occur. Cutting tests or x-rays can be used to determine the number

Table 2—*Regression models with the best fit (highest R^2) for each species*

Species		Best model*	R^2	SE of estimate	No. of seedlots
Sand pine	$\sqrt{G}$	$= 12.8\ [\pm\ 1.13] - 1.42\ [\pm\ .33]\sqrt{x}$	.504	1.17	20
Shortleaf pine	$\sqrt{G^\dagger}$	$= 12.3\ [\pm\ 0.67] - 1.02\ [\pm\ .14]\sqrt{x}$	.504	1.74	51
Virginia pine	G	$= 52.5\ [\pm\ 2.87] + 194\ [\pm\ 29.55]\ 1/x$	.683	6.45	22
Jack pine	$(G)^2$	$= 9329\ [\pm\ 463.4] - 1.00\ [\pm\ .18]\ x^2$	.501	1140	33
Red pine	G	$= 101\ [\pm\ 9.12] - 0.049\ [\pm\ .17]\ x$	.339	16.14	19
Scotch pine	$G^\dagger$	$= 160\ [\pm\ 14.03] - 74.7\ [\pm\ 8.6]\ \log x$	.751	14.02	27

*G = germination percentage; x = conductivity in μS/g. Coefficients of standard deviations are in brackets.
†G includes dormant, abnormal, and empty seeds.

Table 3—*Seed quality classes of five pine species based on leachate conductivity measurements*

Seed quality class	Approximate germination ranges (%)*	Leachate conductivity (μS/g)				
		Sand pine	Shortleaf pine	Virginia pine	Scotch pine	Jack pine†
High	85–100	<6.5	<9	<6	<6	<45
Medium	65–85	6.5–11	9–17	6–15	6–10	45–70
Low	40–65	11–21	17–34	15–40	10–35	>70
Poor	<40	>21	>34	>40	>35	ND

*Includes dormant and empty seeds and abnormal germination for shortleaf and Scotch pines.
†100 seeds to 25 ml deionized water.
ND = no measurements in this range.

of empty seeds, which can then be subtracted from the germination percentage calculated with the model. Depending on the species and lot, 2 or 3% could also be subtracted for abnormal seedlings.

The standard errors are sufficiently large in these models that accurate prediction of germination with conductivity measurements is uncertain. An alternate approach is to use this technique to group seedlots into quality classes. Such a grouping is suggested in table 3 for 5 of the species (excluding red pine).

A major source of variation in this method is the difference among seed sources within species. Pitel's earlier work with accelerated aging of jack pine (S) demonstrates this fact. Accelerated aging treatments are short (2 or 3 days) periods of high humidity and temperature, which simulate natural aging in seeds (2). Pitel aged a single seedlot and paired measurements of leachate conductivity and germination produced an RZ of 0.907, much higher than the 0.501 for jack pine in table 2. Even so, Pitel's data fit very well with the quality classes for jack pine in table 3. The effect of mixed seedlots on model fits has also been noted in our laboratory with spruce (Picea) (4).

It should be emphasized that this test is nondestructive. Another application could be periodic evaluation of seed quality in stored lots. If baseline measurements are taken when fresh seeds go into storage, subsequent rising conductivity values would alert managers that something was wrong. Following measurements, samples can be redried and returned to storage. In this way, quality can be estimated on small but valuable seedlots without sacrificing seeds. The technique could thus be used to track viability decline in extended storage of germplasm conservation collections.

As an example, assume that a measurement on a lot of jack pine yields a conductivity of 40. Seed quality class would be "high" (germination of 85 to 100%) according to table 3. If the regression model or table 1 is used, predicted germination would be 87, with a range of 83 to 92%.

Conclusion

Accurate predictions of viability usually require models of individual seedlots. Most nursery managers or other seed users are not in a position to acquire these models, so they can use table 3 to quickly estimate seedlot quality. Anyone with a conductivity meter can follow the procedures outlined in steps 1 to 7 in this article to arrive at the estimate. Accuracy also can be improved if the percentage of empty seeds in the lot is known.

Literature Cited

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