USING LEACHATE CONDUCTIVITY OF BULKED SAMPLES TO ESTIMATE SEED QUALITY

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Abstract

Conductivity measurements of the leachate of bulked samples with a single-probe instrument offer a simple, completely objective, non-destructive, and inexpensive method to obtain estimates of seed quality for some species in 24 hours. Seeds of six southern species were tested with single-probe meters. Standard errors of estimate of about 9 percent were obtained in laboratory germination tests of loblolly and slash pines. Regressions for longleaf pines and sweetgum were also significant, but predictions were not as accurate. Results for shortleaf pine and sycamore were unsatisfactory. Additional research on methodology should improve the accuracy for all of these species.

Additional keywords: Pinus, Liquidambar, Platanus, southern pines.

One of the earliest reports of the correlation between germination of tree seeds and the substances that leached from them was that of Hocking and Etter (1969) for white spruce (Picea glauca). Similar relationships were later reported for sugar pine (Pinus lambertiana) (Murphy and Noland 1982); jack pine (P. banksiana) (Pitel 1982); Norway spruce (Picea abies) (Schindlbeck 1981); longleaf (P. palustris) and spruce (P. glabra) pines (Barnett 1985); and loblolly (P. taeda), slash (P. elliottii), eastern white (P. strobus), longleaf, shortleaf (P. echinata), and Virginia(P. virginiana) pines (Bonner 1986, Vozzo and Bonner 1986). In all of this research, the amount of leached components increased as the seeds deteriorated. The work on southern pines utilized the multiple-probe seed analyzer ASAC-1000, manufactured by Neogen, Inc. Early results were very promising, but repeated problems with calibration of the ASAC-1000 negated the original conductivity/germination relationships developed in our laboratory. The high cost of this instrument also discouraged adoption of the technique.

Despite these problems, the relationship of leached cellular constituents to seed quality *is* apparently biologically sound, and it is the most objective of all rapid measurements of seed quality. The International Seed Testing Association (ISTA) (1981) endorses the use of single-probe conductivity meters with bulked samples for estimates of seed quality in agricultural seeds. Following their methodology, we have evaluated single-probe meters in our laboratory with several southern tree species.

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[1] The *use* of trade or firm names in this publication is for reader information only and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

Materials and Methods

Preliminary Tests

To evaluate optimum sample size, 10 seed lots of loblolly pine were selected, and samples of 25, 50, and 100 seeds were drawn from each. Each sample was placed in a 100-m1 beaker, and 100 ml of deionized water was added. Electrical conductivities were measured with a Markson ElectroMark conductivity meter after 1, 2, 4, 6, 8, 12, 24, and 32 hours.

Concern over the need for agitation of bulk samples led to selection of paired samples of 100 seeds each from 20 randomly selected loblolly lots. The samples were placed in 100-ml beakers to which only 90 ml of deionized water was added; the samples remained there for 24 hours. One set of samples remained stationary, while each beaker in the other set was continously stirred with a teflon-coated pellet on a magnetic stirrer. Conductivity was read as before, and a "t" test was used to determine differences.

In the first test with loblolly pine, two conductivity meters were compared: the Markson and YSI Model 32. Readings were taken with both meters on over 60 samples.

Calibration Models

Seed lots on hand at the Forestry Sciences Laboratory, Starkville, Mississippi, were used to develop calibration models for the following species: loblolly, slash, longleaf, and shortleaf pines; sweetgum *(Liquidambar styraciflua);* and sycamore *(Platanus occidentalis).* Seventeen to 20 lots of each species were used. Empty seeds were removed by water flotation (loblolly pine and sweetgum) or with laboratory blowers (all other species). Procedures from the ISTA Vigor Testing Handbook (International Seed Testing Association 1981) were generally followed. Duplicate samples of 100 seeds were counted out and placed in laboratory beakers: 250 ml for longleaf and 100 ml for all others. Broken or crushed seeds, wings, or other small trash were not included. The samples were weighed to three significant figures, and deionized water was added: 95 ml for 100-m1 beakers and 200 ml for 250-m1 beakers. Each sample was stirred with a spatula, covered with a petri dish half, and placed in a dark germinator constantly maintained at 20+1°C. Two beakers containing deionized water, but no seeds, were also placed in the germinator.

After 24 hours, the beakers were removed, and the seeds were strained from the leachate. Electrical conductivity was determined for all leachates and the water blanks. The seeds were saved for laboratory germination tests that were conducted according to the Association of Official Seed Analysts (1981) standards.

Conductivity readings were expressed as micromhos g^{-1} after subtraction of the water blanks. Germination values were regressed on conductivity using MINITAB routines on a DG MV 4000 computer. Many transformations, as well *as* second- and third-order polynomial regressions, were tested to obtain the best fitting models. The germination values for most of the species include dormant and empty seeds and abnormal seedlings, as well as the normal seedlings, because conductivity measurements cannot distinguish between them (Vozzo and Bonner 1986).

Results and Discussion

Preliminary Tests

As expected, conductivity increased with sample size (Figure 1). The 100-seed sample, which is the traditional size for all seed testing, was adopted for *use* because the range in values between the best and worst lots was large enough for logical separations. Constant stirring did not significantly change conductivity readings. The means were 24.6 and 25.4 micromhos g for non-stirred and stirred samples, respectively (t = 0.447, n = 20).

Below 30 micromhos g^{-1} conductivity, there was little difference between meters (Figure 2). Only at very high levels was there a difference, and few samples read that high. Because the YSI meter had automatic temperature compensation, it was used in all subsequent tests.

Calibration Models

The single-probe method worked well with some **species**, but not so well with others (Table 1). The best correlations between leachate conductivity and laboratory germination were obtained for loblolly, slash, and longleaf pines and sweetgum (Figures 3-6). Poor correlations were obtained for shortleaf pine and sycamore (Figures 7 and 8).

Estimates of laboratory germination within + 10 percent are certainly acceptable for simple, objective, rapid (24-hour) measurements. Even if nursery managers only use the technique to separate lots into three classes of seed quality (high, medium, or low), the method can be very useful. Many circumstances occur when germination must be evaluated, yet there is not enough time for a 28-day germination test (56 days if stratification is required). The method can be used as a non-destructive test on small seed lots from breeding programs and also to check deterioration of seeds in long-term storage. Of course, seeds would have to be re-dried to be returned to storage. This application could be very useful in germplasm conservation programs, where periodic standard germination tests could require large amounts of seeds over a long period of time.

Can accuracy be improved? Refinement of laboratory techniques could probably help some. A standard rinse to remove external contaminants is one possibility; increasing sample size is another. The latter suggestion could be important for small seeds, such as shortleaf pine and sycamore. There is considerable evidence that electrolyte leakage is positively correlated with seed size (Vozzo and Bonner 1986). Four 100-seed samples would probably be better than two, just as in germination tests.

Another practical step to improve accuracy would be to delete all lots having germination below 50 percent from the calibration models. Regressions based on the better lots will have much smaller errors of estimate (Bonner 1988); in any case, lots below 50 percent germination are seldom, if ever, planted. Conductivity readings above a maximum level would indicate a seed lot too poor to be used, and germination estimates on the better lots would be improved.

Estimates would also be better if no empty seeds were in the seed lots. Although this is normally the case in good seed management, some lots do contain empty seeds. To counter this, an estimate of empty seeds can be obtained with cutting tests, or better still, with an X-ray before leaching. This value is then subtracted from the conductivity prediction to give improved estimates. Some nurserymen might also subtract a few percentage points for dormant seeds in loblolly or other species, or for abnormal seedlings in lots known to have this condition.

All of these steps could help improve the accuracy of germination estimated from conductivity readings, and other steps will undoubtedly occur to users. Additional tests are already underway in our laboratory at Starkville. The low cost and simple methodology of the bulked sample technique make it very attractive. For most seed lots, inexpensive conductivity meters, such as the Markson, should do the job. They currently (1988) cost less than $500, about one-third of the cost of the YSI meter and 3 percent of the cost of an ASAC-1000.

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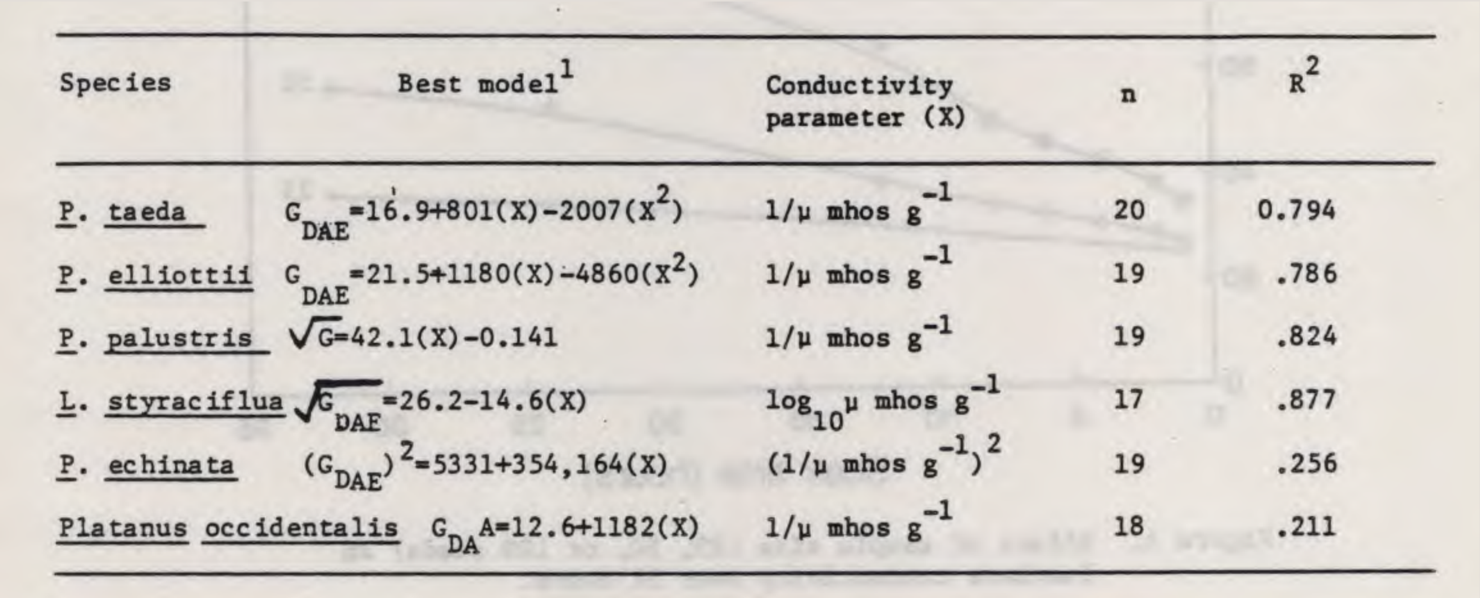
Table 1. Calibration models with the best fit for each species.



Species	Best model[1]	Conductivity parameter (X)	n	R^2
P. taeda	$G_{DAE}=16.9+801(X)-2007(X^2)$	$1/\mu$ mhos g^{-1}	20	0.794
P. elliottii	$G_{DAE}=21.5+1180(X)-4860(X^2)$	$1/\mu$ mhos g^{-1}	19	.786
P. palustris	$\sqrt{G}=42.1(X)-0.141$	$1/\mu$ mhos g^{-1}	19	.824
L. styraciflua	$\sqrt{G_{DAE}}=26.2-14.6(X)$	$\log_{10}\mu$ mhos g^{-1}	17	.877
P. echinata	$(G_{DAE})^2=5331+354{,}164(X)$	$(1/\mu$ mhos $g^{-1})^2$	19	.256
Platanus occidentalis	$G_{DA}A=12.6+1182(X)$	$1/\mu$ mhos g^{-1}	18	.211

[1] Expressions of germination:

G = normal germination only; G_{DAE} includes dormant and empty seeds, plus abnormal seedlings; G_{DA} includes dormant seeds and abnormal seedlings.

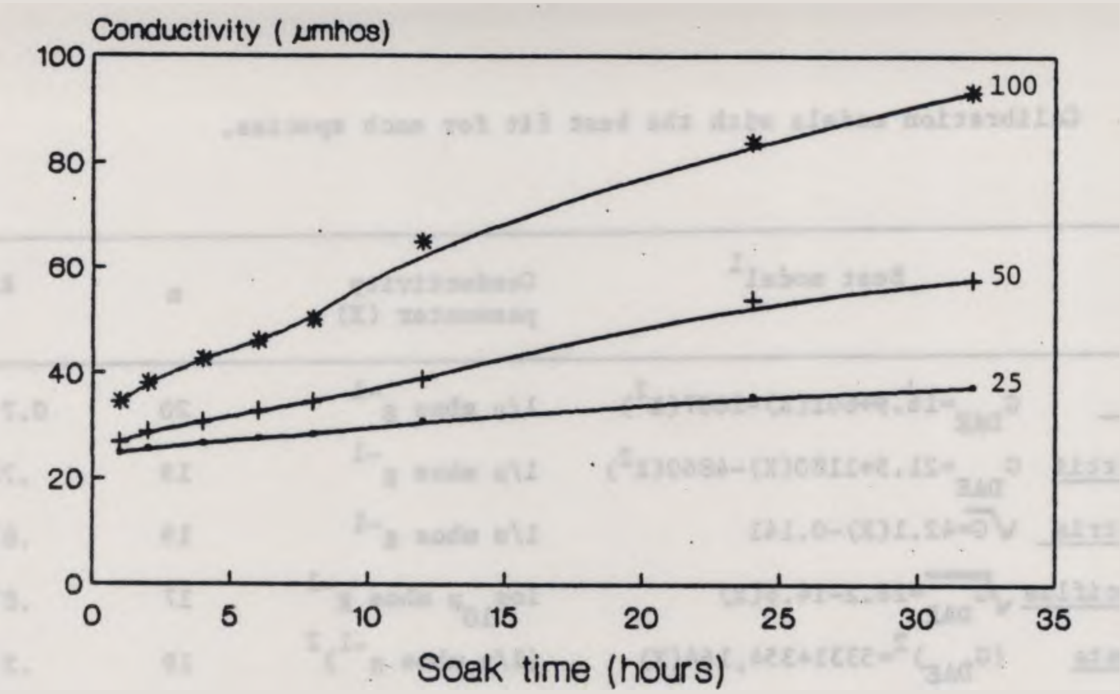


Figure 1. Effect of sample size (25, 50, or 100 seeds) on leachate conductivity over 32 hours.

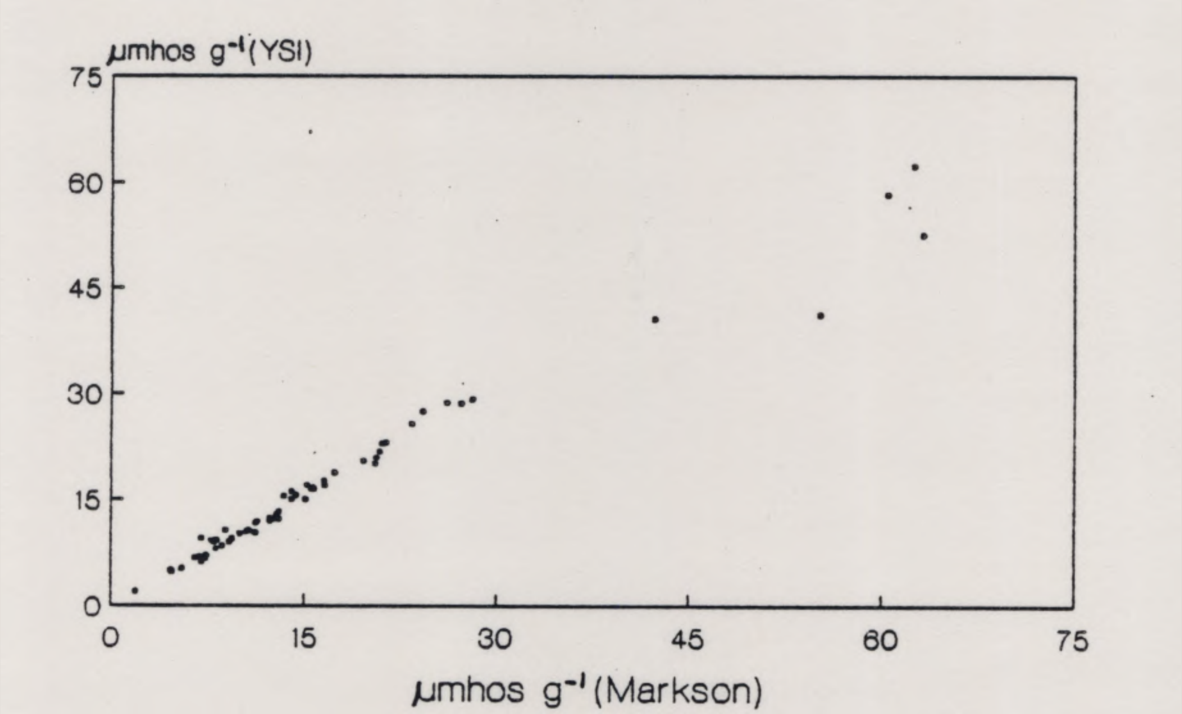


Figure 2. Comparison of YSI and Markson conductivity meter readings (n=60).

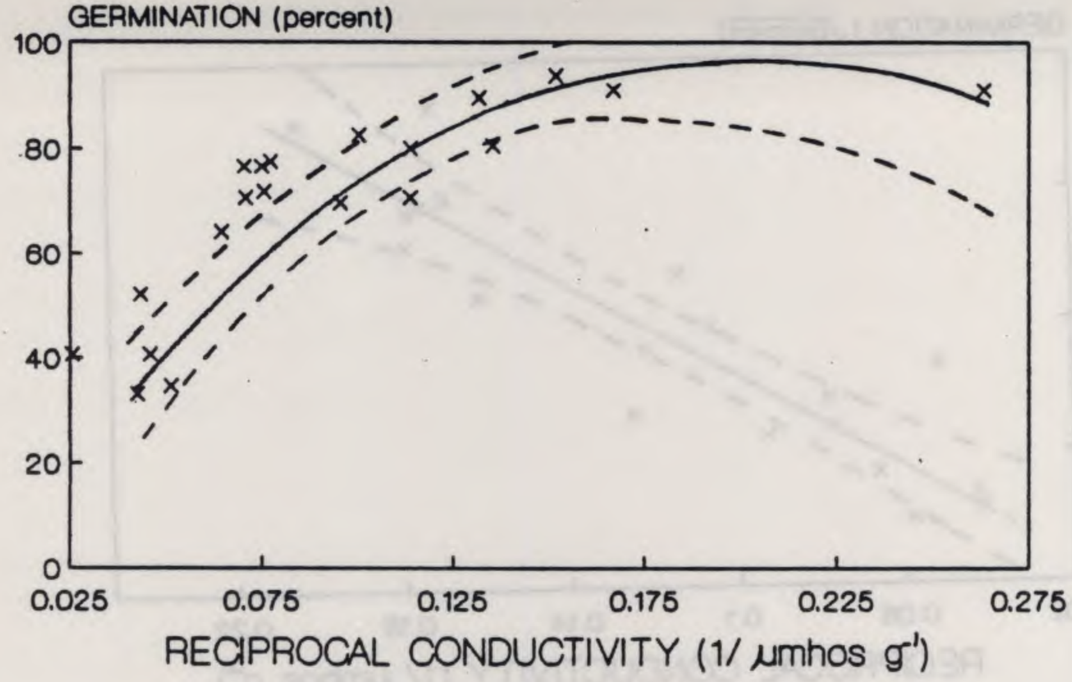


Figure 3. Relationship of leachate conductivity to laboratory germination of loblolly pine. Germination includes dormant and empty seeds and abnormal seedlings. Confidence limits: 95%.

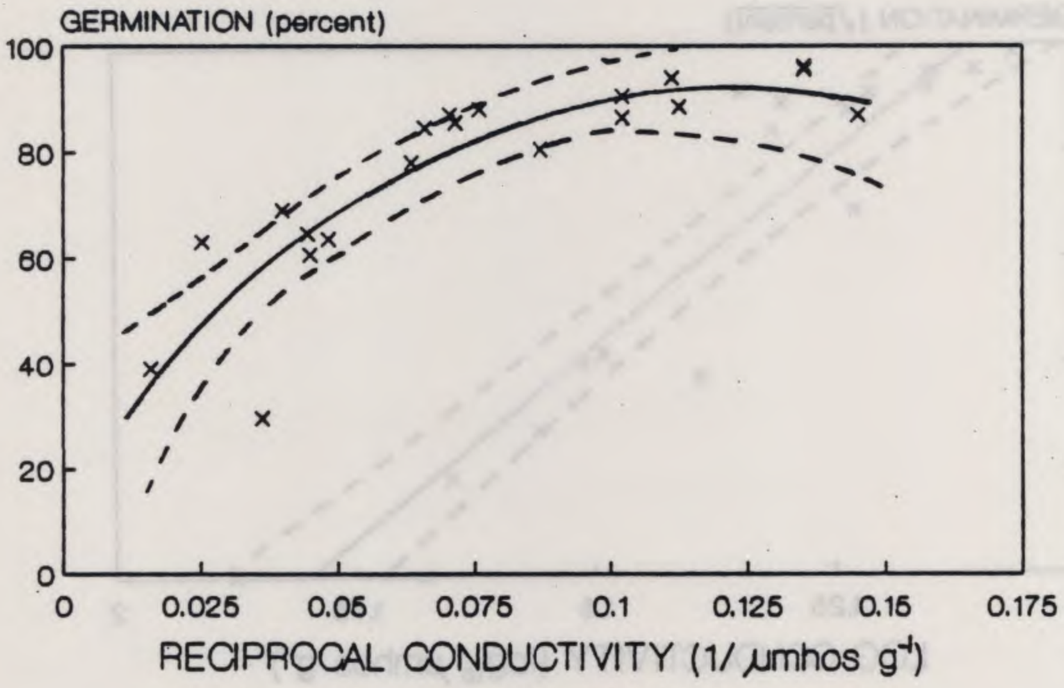


Figure 4. Relationship of leachate conductivity to laboratory germination of slash pine. Germination includes dormant and empty seeds and abnormal seedlings. Confidence limits: 95%

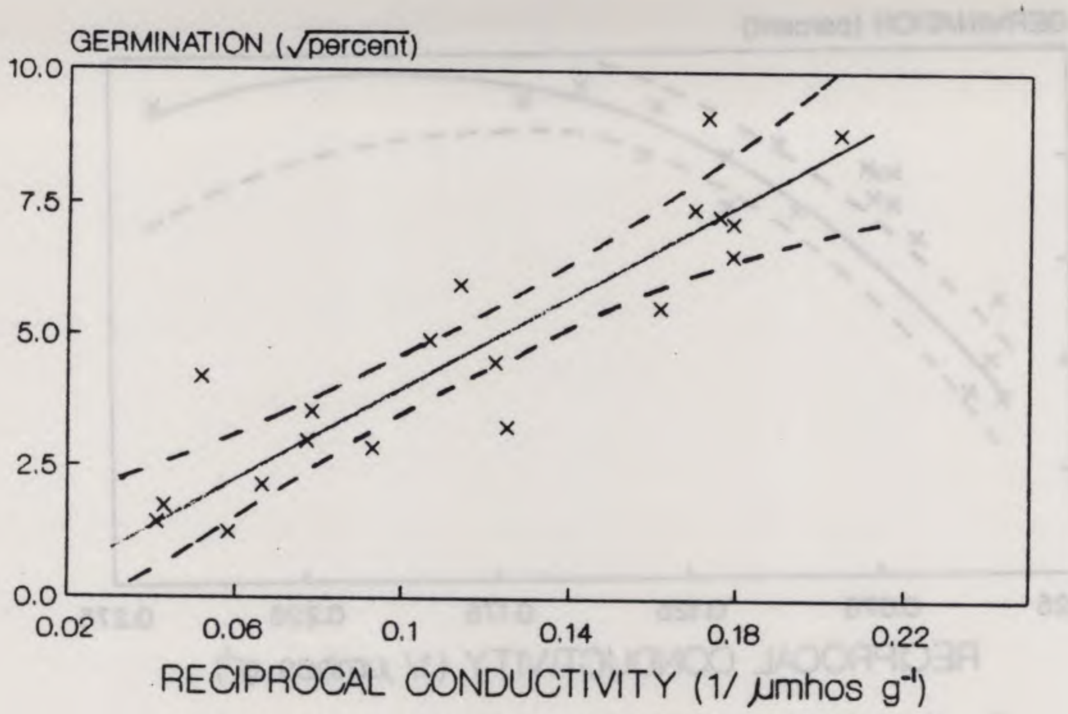


Figure 5. Relationship of leachate conductivity to laboratory germination of longleaf pine. Germination includes normal seedlings only. Confidence limits: 95%

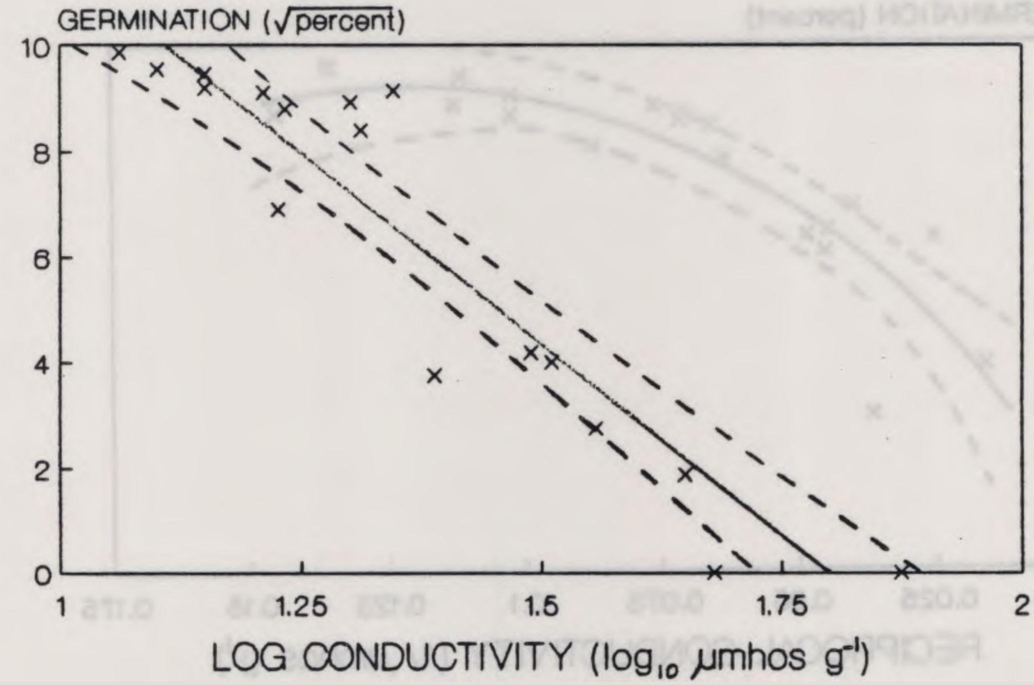


Figure 6. Relationship of leachate conductivity to laboratory germination of sweetgum. Germination includes dormant and empty seeds and abnormal seedlings. Confidence limits: 95%

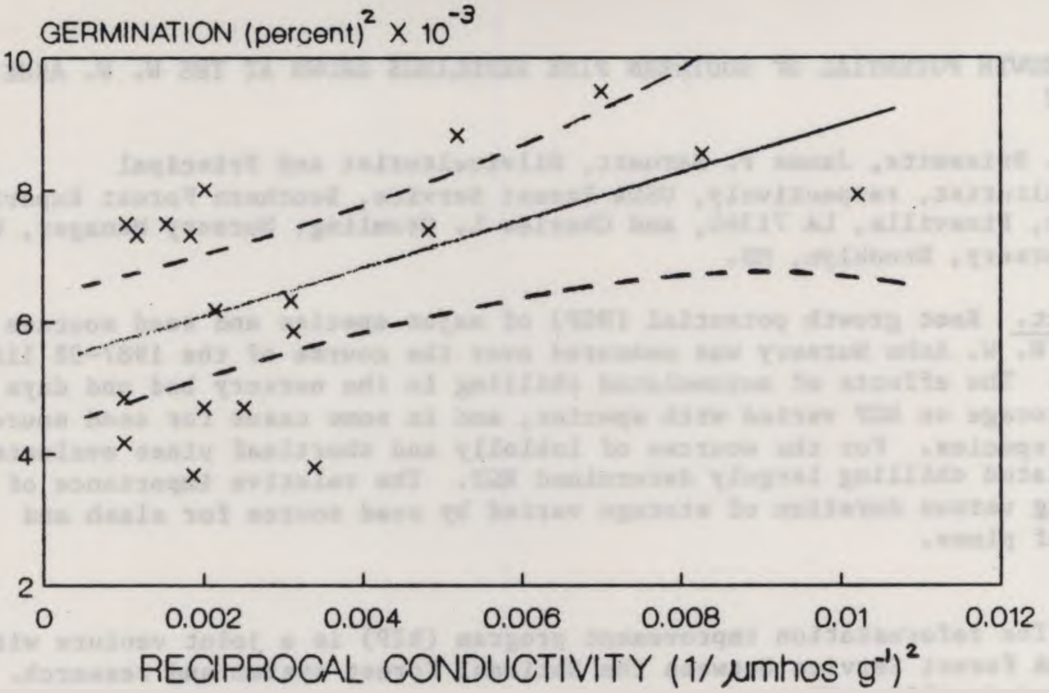


Figure 7. Relationship of leachate conductivity to laboratory germination of shortleaf pine. Germination includes dormant and empty seeds and abnormal seedlings. Confidence limits: 95%

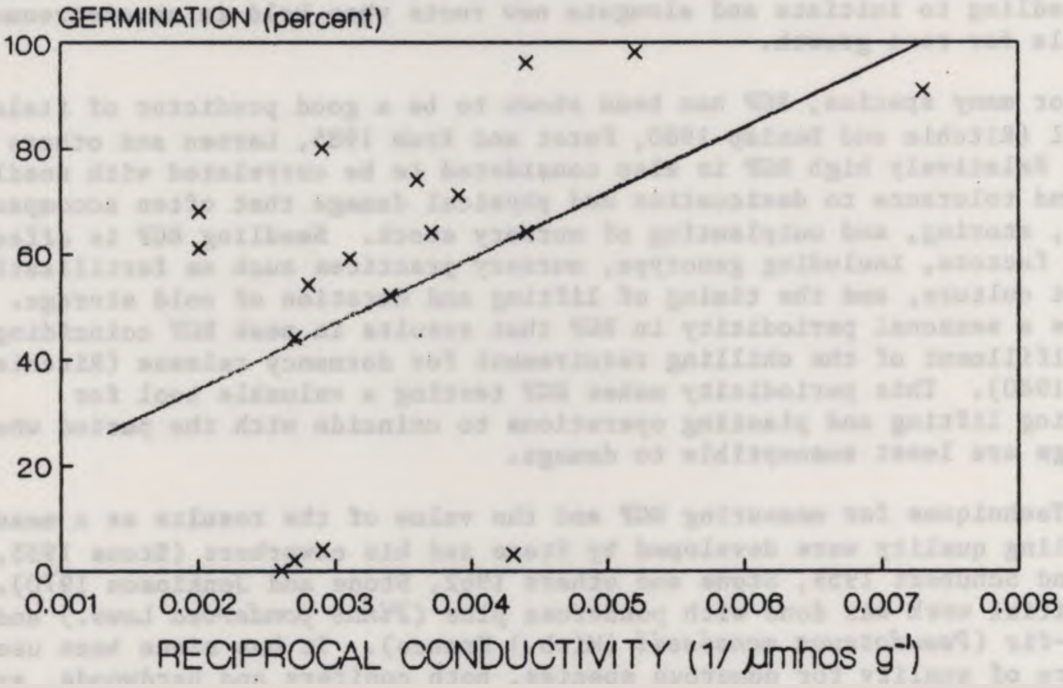


Figure 8. Relationship of leachate conductivity to laboratory germination of sycamore. Germination includes dormant (good ungerminated) seeds and abnormal seedlings.