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Use of the Richards Function to Interpret Single Seed Conductivity Data

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Abstract. **Previous research showed a relationship between viability and electrolyte leakage; here, we develop an index of viability based on electrolyte leakage from sample populations of seeds soaked in deionized distilled water. Conductivity of leachates from individual seeds was determined for 10 lots of lettuce (*Lactuca sativa* L.), each germinating at 99%. Conductivity data for two lots of soybean (*Glycine max* L.) seeds germinating at 100% and 74%, respectively, were obtained from literature. Cumulative frequency distributions (CFD) with a class interval of one μA, were fitted with a natural logarithmic form of the Richards function, which requires no arbitrary starting values. The procedure provided an effective estimation of slopes [$(dCF/d\mu A)_{MAX}$] of hypothetical lines tangent to inflection points of the respective sigmoidal CFD curves. We suggest that this maximum slope, or *internal slope* can be used as a seed viability index. The index is unaffected by outlier μA readings and reflects the shape of the CFD. It is also a measure of seed-to-seed variability in leachate conductivity. The SE of the 10 internal slopes derived from the 10 lettuce seed lots was 3.8. The viability index is sensitive, since nearly a four-fold difference in internal slope was found for the two soybean seed lots. The greater the internal slope, the less the variation among individual seed conductivities and the higher the seed quality.**

Conductivity of seed leachate is related inversely to germination (10) and emergence (4). Parrish and Leopold (6) noted an increase in leachate conductivity prior to a decline in seed viability, suggesting an association with a decline in seed vigor. Their evaluations were based on measurements of bulked samples of six cotyledons each. An instrument is now available that measures conductivities of single seed leachate. Steere et al. (10) used a "histogram segment" analysis to estimate seed quality based on samples consisting of 100 individual seed leachates. Their method is a subjective attempt to incorporate shape of a cumulative frequency histogram into an index of seed quality. Here, we suggest a more objective parametric analysis of the cumulative distribution of leachate observations, one that retains the idea of shape.

Cumulative frequency distributions (CFD) are constrained to be sigmoidal with a slope that is relatively constant between inflections. This slope, a maximum, is approximated by the slope of a line tangent to the inflection point of the CFD curve. It may be generalized by $(dY/dX)_{MAX}$; we call it the *internal slope*. This application is in keeping with the term *initial slope* used by Milthorpe and Moorby (5) to refer to dY/dX_{MAX} of nonsigmoidal curves.

Our present objective was not to relate conductivity measurements to viability per se, but to develop an index of viability based initially on the frequency distribution of current carrying capacity of leachates from individual seeds in the sample population. Ultimate benefit would be elimination of the labor-intensive standard germination test (2).

Ten lettuce (*Lactuca sativa* L.) seed lots, each with 99% germination, were selected from storage. From each lot, seeds, one seed per cell, 100 cells at a time, were soaked in deionized distilled water for 24 hr at 22°C. Electrical current (μA) conducted by the imbibing solution for each 100-seed sample then was measured using an automatic seed analyzer set at 4 V (ASA 1000, Neogen Food Tech Corp., Lansing, Mich.). The lettuce data were ordered into frequency classes from which cumulative frequency distributions were generated. In addition, cumulative frequency polygons were constructed for two soybean (*Glycine max* L.) seed lots from cumulative frequency histograms presented as figures in the instructions manual for the instrument. The soybean seed lots were reported to have 100% and 74% germination, respectively. Leachate observations [i.e., cumulative frequency (CF) and μA values] were digitized from the polygons.

Cumulative frequency observations were paired with observations of successive μA classes, each having a class interval of 1 ($n - 1, n, n + 1$). Primary data consisted of class interval observations (X), which were integer values greater than zero, and a minimum CF (Y) arbitrarily was initialized at 0.1 to facilitate curve fitting.

Inspection of cumulative frequency histograms produced previously by the seed analyzer revealed monotonic trends that rose rapidly and quickly approached upper asymptotes in the case of high-quality seeds. A steep internal slope was noticeable for high-quality seeds. Internal slopes were derived for the 10 lettuce and two soybean seed lots. We first fitted a version (7, 11) of the flexible (Fig. 1) Richards (8) function to the relationship between cumulative frequency (Y) and μA class (X):

$$\ln(Y) = \ln(A) - \ln\{1 \pm \exp[-B(X - D)]\}/C \qquad [1]$$

A, B, C, and D are constants that control the form (Fig. 1) of the function and were estimated by the curve fitting program (Division of Mathematics and Statistics, CSIRO, Canberra, Australia).

Using the sigmoidal CFD data sets from the 10 lettuce seed lots, we tested how the estimated internal slope approximated the slope of the straight line segment of the 10 sigmoid curves. Data subsets were selected objectively (3) for their approximation of internal straight line segments. An algorithm for second derivative (change in the rate of change in Y) interpolation of Eq. [1] was included in the Richards fitting program in order to locate objectively the straight line segments that spanned the inflection points of the previously described 10 CFDs. Each of the selected subsets was fitted by a first-order polynomial and the regression lines were plotted (Fig. 2).

Average $(dY/dX)_{MAX}$, in this case $(dCF/d\mu A)_{MAX}$, determined from the Richards parameter estimates was 22, and the average b value estimate, based on the internal straight line segments, was 20; each method produced about the same SE (Table 1). Line segment slope and internal slope were proportional.

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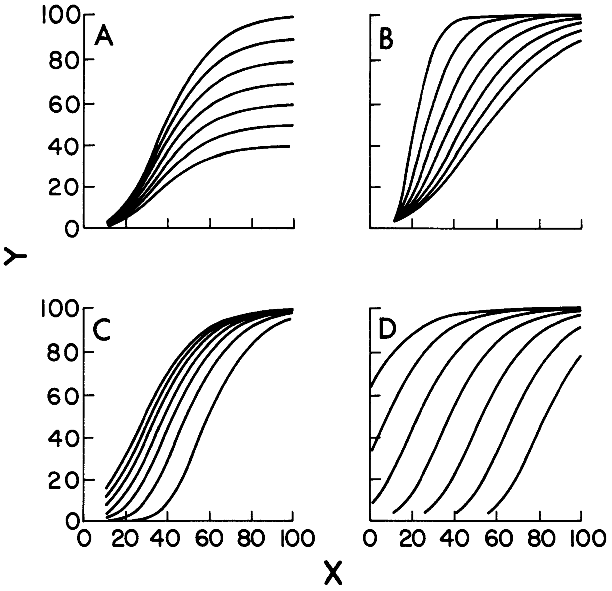

Fig. 1. Example. Flexibility of the Richards function $\ln(Y) = \ln(A) - \ln\{1 \pm \exp[-B(X-D)]\}/C$ is demonstrated by successively holding three of the four parameters constant and varying the fourth. In A; B, C, and D were held constant and in B; . . . etc.

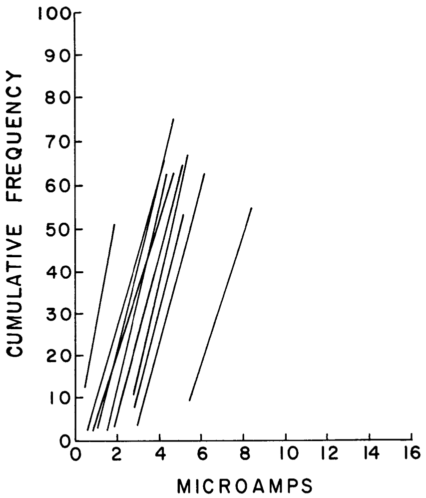

Fig. 2. Experimental. Line segments between inflections within sigmoidal cumulative frequency distribution curves for 10 lettuce cultivars each germinating 99%.

Standard error of internal slopes for lettuce was found to be 3.8 (Table 1), whereas SE of laboratory germination tests, assuming a binomial distribution, is 1.0 at 99% viability and a sample size of 100 seeds (1).

The approach suggested here incorporates shape of the CFD into a seed quality index. Also, it appears to be sensitive, based on the soybean data fit (Fig. 3), from which the ratios 100% : 74% vs. 5.8 : 1.6 were derived. The magnitude of the difference in the two internal slopes, 5.8 and 1.6, quantifies our visual approximations of cumulative frequency histograms output by the seed analyzer pertaining to high- and low-quality seeds, respectively.

Fitting the Richards function would suppress bimodality if it occurred; however, small sample size (e.g., 100 seeds) is dictated; this factor alone would suppress evidence of bimodality. Although fitting the natural logarithmic form of the Richards function may reduce failures to converge, upper limbs may not fit observations well (Fig. 3). A statistical remedy would be the application of the proper weighting function to the data.

Most often, arithmetic means, medians, and sometimes modes are used to describe populations and, in this case, would qualify as indicators of seed quality; however, means are affected strongly by extreme values and, furthermore, all three indices are measures of distribution location (9). Internal slope is not associated with distribution location and therefore has wider application. Our approach accommodates normal as well as nonnormal data distributions (i.e., skewed data) and, although parametric, it has the advantage of not requiring the usual arbitrary choice of initial parameter values.

Seed samples from seed populations of low viability exhibit much variation in times to germination with regard to individual seeds. We suspect that this phenomenon is reflected by variation in ion leakage on a seed-to-seed basis and that it is summarized by internal slope. Figure 4 presents an example, albeit based on a normal distribution, which shows how internal slope is related to standard deviation of a random variable, in this case μA, which indicates magnitude of variation in ion leakage among seeds in a sample population.

We have developed an index to be used to estimate seed viability. The internal slope of the CFD curve of the ion leakages reflects the shape of the CFD curve. The shape of the CFD curve is related to the slope of the straight line segment of the curve. This slope is proportional to the slope of a line tangent to the inflection point of the CFD curve. The latter is, therefore, a maximum $(dY/dX)_{MAX}$, i.e., $(dCF/d\mu A)_{MAX}$. We call this maximum "internal slope", an index of seed viability. The index has physiological as well as mathematical bases. As with any parametric method, however, failure of the initial regression, due to insufficient or poor data causing data/model incompatibility, would preclude the use of the index.

Table 1. Experimental. Primary data are cumulative frequency paired with μA per cell, with each cell containing one seed and its leachate. Segment slope is derived from first order polynomial fit, and internal slope is derived from Richards fit.

j	Sample viability	Segment slope b (Y_1j)	Internal slope $(dY/dX)_{MAX}$ (Y_2j)	$Y_2j - Y_1j$
1	99%	18.2	20.2	+ 2.0
2	″	20.8	23.0	+ 2.2
3	″	19.7	21.8	+ 2.1
4	″	18.7	20.6	+ 1.9
5	″	15.9	17.5	+ 1.6
6	″	22.3	24.5	+ 2.2
7	″	17.1	19.1	+ 2.0
8	″	15.8	17.4	+ 1.6
9	″	27.2	30.2	+ 3.0
10	″	19.7	22.0	+ 2.3
N		10	10	
Y		19.5	21.6	
SE		3.4	3.8	

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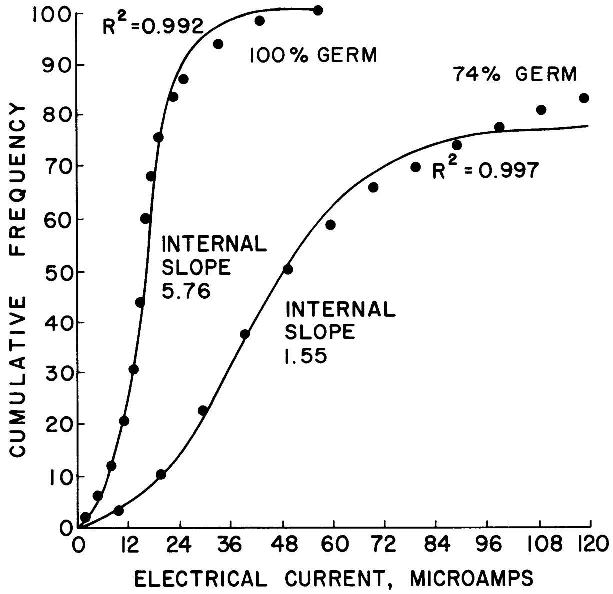

Fig. 3. Experimental. Cumulative microamp frequency observations for soybean. Solid lines were estimated from fitting the Richards function. Primary data were supplied by the Neogen Food Tech Corp., Lansing, Mich.

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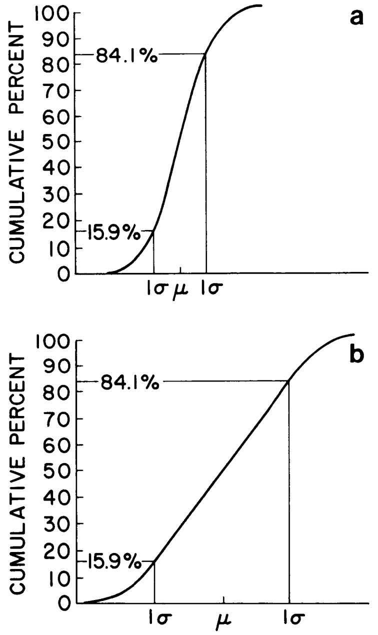

Fig. 4. Example. Cumulative frequency distributions which are normal showing how reduction in standard deviation, σ, is associated with increase in *internal slope.* Note the steeper slope in **a** resulting in a smaller standard deviation relative to **b**, which exhibits a less steep slope and therefore a larger standard deviation.