THESIS

INDIVIDUAL SEED ELECTROLYTE LEAKAGE TESTS AND EVALUATION OF SOAKING INJURY USING MAIZE

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY KIMBERLY V. DAVIDSON ENTITLED **INDIVIDUAL SEED ELECTROLYTE LEAKAGE TESTS AND EVALUATION OF SOAKING INJURY USING MAIZE** BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

INDIVIDUAL SEED ELECTROLYTE LEAKAGE TESTS

AND EVALUATION OF SOAKING INJURY USING MAIZE

Determination of seed viability has traditionally involved germination, which is obviously destructive to the seed and also labor intensive. Both are far from being cost effective. The development of non-destructive or at least less injurious methods of testing seed quality i.e. viability and vigor determination using electroconductivity measurements of single seed leachate solutions could effectively replace the standard germination test. The objectives were to compare five indices of seed quality, all of which are based on individual seed leachate conductivity tests. Additionally, if the soak period is brief enough, there should be little injury to the seeds allowing for successive soaks on the same sample.

Seeds of *Zea mays* L. were aged using two methods to obtain varying levels of viability and vigor for comparisons. The first method, modified controlled deterioration, involved placing two samples of seeds in separate desiccators over a saturated NaCl solution for 16 and 20 days for seed lots 88-2i and 88-1d, respectively. The seeds were sealed in aluminum foil packets, 110 per packet, and aged for 120 hours at 45°C. The second method involved placing two samples of seeds in separate desiccators over H_2SO_4 at 35°C for 238 and 484 days, respectively for desiccators one and two. After aging, seeds from each desiccator

were kept in aluminum foil packets. Electroconductivity testing was done on samples of 100 seeds from each of the aging methods. The seed samples were soaked for six hours during which time 29 scans of data were obtained. The samples were dried at room temperature for seven days after which time they were germinated using the rolled paper towel method. An additional 100 unsoaked (control) seeds were germinated at the same time. Radicle lengths were measured at the end of 72 hours and final germination was counted at the end of seven days. Relative vigor was calculated as a ratio of the soaked seed radicle lengths divided by the unsoaked control seed radicle lengths.

Electroconductivity data were concatenated and made compatible with the UNIX format. Five indices were derived from the data for determination of their ability to predict maize seed quality. Internal Slope (IS) and mean and median μAmps after five hours of imbibition were derived from a Richards function program, the Initial Leach Rate (ILR) was derived from the rectangular hyperbola and the Average Absolute Leach Rate (AALR) was derived from another Richards function program.

The second aging method did not produce the desired range of seed quality and so the results discussed are based on the first aging technique. Internal Slope was the best predictor of seed viability, $r^2 = 0.91$, followed very closely by the median μAmp value, $r^2 = 0.87$, and the mean μAmp value, $r^2 = 0.81$. The ILR and AALR indices did not predict seed quality with r^2 values of 0.01 and 0.03, respectively. Relative vigor was not estimated as well as viability, probably due to the artificial aging.

A second experiment was designed to study the effect of five successive soak cycles (C) and five cycle durations (CD) of 2, 4, 6, 7 and 8 hours on viability and vigor loss response. All subsets regression plus consideration between bias and random error led to the choice of the following two best subset models: $Y_{VIA} = 99.14 - 0.0609\ (CD*C^2)$, $R^2 = 0.62$, and $Y_{RV} = 0.99 + 0.0229\ (CD) - 0.0101\ (CD*C)$, $R^2 = 0.52$. Response surfaces were generated which suggested that 4 C of 5 hours each resulted in only an 8% loss of viability but a 20% loss of relative vigor. Conductivity measurements taken at the end of each CD for each C showed that 45% of the readily leachable electrolytes leached during the first soak period. Furthermore, a priming effect, invigoration, was observed when the seeds were soaked for a total of ten hours, taking into consideration both the number of cycles and the duration of each cycle.

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For my father, C. E. Vanderpool,
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INTRODUCTION

Determination of seed viability has traditionally involved germination, which is obviously destructive to the seed and also labor intensive. Both are far from being cost effective. Very often quantities of seed available for testing are limited, therefore, it is important to develop a method to test a seed sample without destroying it. The development of non-destructive or at least less injurious methods of testing seed quality, i.e. viability and vigor determination, using electroconductivity measurements of single seed leachate solutions could effectively replace the standard germination test.

Seed injury is expressed as membrane leakage. The electroconductivity test is based on the principle that the amount of seed deterioration is expressed by a proportional loss of cell membrane integrity. For example, a non-viable seed leaches more electrolytes than a healthy, viable seed. The earlier in the soak period these tests can be done, presumably, the less injury occurring to the seed and therefore, the less destructive the test.

The early approaches using electroconductivity involved long soak times, bulked seed samples and data partitioning. A partition value in terms of current carrying capacity is determined empirically and assumes that all seeds with values above the partition value are dead and all seeds with values below are alive. All three of the above methods are rejected here on theoretical grounds. A non-central tendency measure of seed viability based on 100 individual seed leachate

conductivities, therefore a total frequency of 100, has been developed to describe levels of seed deterioration (Moore *et al.*, 1988). It is called 'Internal Slope'.

Although internal slope, IS, has potential as a predictor of viability and its' reciprocal has biological relevance it seemed that leakage rates averaged over the entire period and initial leakage rates should also be investigated. Measures of additional frequency distribution derivates such as trimmed mean μAmps and median μAmps should be considered as well. Methods of aging such as accelerated (Delouche and Baskin, 1973), controlled deterioration (Bruggink, 1989) and artificial (Vertucci and Roos, 1990) to obtain different levels of viability with which to test the candidate indices were examined. Furthermore, it was necessary to ascertain seed dryback tolerance to determine the destructiveness of the electroconductivity test, which requires soaking. Thus, it was essential to determine viability and vigor loss associated with soaking.

The goal here was to investigate possible indicators of maize seed quality using electroconductivity testing of one hundred individual seed leachates. More specifically, the objectives were to use maize seeds (*Zea mays* L.) and compare IS with other candidate indices for evaluating seed lots and to study the effect of repeated imbibition/dryback cycles (C), cycle duration (CD) and seed moisture (SM) levels of 10% and 18% (between drybacks) on seed viability and vigor.

LITERATURE REVIEW

Theory of Electroconductivity Testing

Conductivity tests have been proposed as seed quality tests for over 20 years. Solute leakage occurs from all seeds when they are placed in water (Powell, 1986). One of the most positively correlated indicators of seed vigor and germinability is the leakage of various intracellular substances from imbibing seeds (Duke *et al.*, 1983). Conductivity of seed leachate has also been found to be a good indicator of seed deterioration (Ching and Schoolcraft, 1968). However, as presently conducted, conductivity tests are similar to the germination test in that they are destructive. The early approaches using electroconductivity (Matthews and Bradnock, 1968; Bondie et al., 1979; McDonald and Wilson, 1980; Furman *et al.*, 1987; Pandy, 1988) involved long soak times, bulked seed samples and data partitioning. At the beginning of the last decade conductivity measurements of individual seed leachates became possible and a data manipulation method for predicting germination was developed (Steere *et al.*, 1981) based on 'partitioning' of μAmp values from the Neogen[1] seed analyzer. The ASAC-1000B (Automatic Seed Analyzer Computer) measures the soak water current carrying capacity, μAmps/seed, which is highly correlated with conductivity, μmhos/seed (McDonald and Wilson, 1979). In the case of a seed, current is carried by ions and ionic compounds leached from the seed into the soak water. The greater the current

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[1] Neogen Food Tech. Corp., 620 Lesher Place, Lansing, Michigan, 48912, USA.

carrying capacity of the leachate, the greater the concentration of ions leached from the seed. Non-viable seeds readily leak electrolytes (Matthews and Bradnock, 1968). Single seed leachate analysis using the ASAC is more accurate at low ionic concentrations (KCl below 10^{-3}Molar) than the bulk method (Hepburn *et al.*, 1984).

Bondie *et al.* (1979) present a concise series of equations relating current flow in the exudate solution to electrical conductivity where the current, *i*, flowing in a volume of material is given by ohms law:

$$i=\frac{V}{R}amperes \qquad (1)$$

Where *V* is the electrical voltage applied across the material volume and *R* is the electrical resistance of that volume of deionized water and seed exudate. The resistance, *R*, can be expressed as:

$$R=\frac{d}{\sigma A}ohms \qquad (2)$$

where *d* is the distance between electrodes, *A* is the cross sectional area of the electrodes and σ is the intrinsic electrical conductivity of the water plus the exudate. The electrical conductivity,σ, is expressed as

$$\sigma=ne\mu(cm)^{-1}Mhos \qquad (3)$$

where *n* is the number of ions in the water between the electrodes, *e* is the electrical charge of the ions and μ is the ion mobility, i.e. the ease with which the

ions move through the solution. Combining equations **(1)** through **(3)** yields the following relation between the electrical current, *i*, and the electrical conductivity, σ:

$$\sigma = [\frac{d}{A}][\frac{1}{V}]i \qquad \textbf{(4)}$$

Equation **(4)** shows that the electrical conductivity, σ, is directly proportional to the electrical current flow, *i*. The first term, *d/A*, in equation **(4)** is a geometrical one which remains the same from one measurement to another as long as the voltage remains constant. The second term, *1/V*, is the inverse of the applied voltage. Seed current measurements made with the ASAC-1000B are not conductivity measurements per se, as expressed earlier. However, *conductivity* is pervasive throughout the literature and will be used throughout this thesis.

Higher germination rates, associated with more vigorous and uniform seedling growth, correspond to lower current levels or conductivity values (Steere *et al.*, 1981). Conductivity measurements of single seed leachates seek to quantify the extent of membrane disorganization by measuring the amount of electrolytes released into the soak water (Pandey, 1988). An important advantage to the measurement of single seed leachate conductivities, versus the bulk conductivity method, aside from the fact that they allow the freedom to study the distribution of current values in the sample population, is that a few severely deteriorated seeds in a bulk test may release a greater amount of electrolytes (thus prejudicing the outcome) than twice as many seeds just at the point of viability loss since seed tissues deteriorate beyond the point of viability loss (Steere *et al.*, 1981).

Conductivity measurements are rapid and simple (Matthews and Bradnock, 1968; Tracy and Juvik, 1988) and as such provide the seed industry with an alternative to the standard germination test, which generally takes as little as seven days and as much as forty two days to complete.

Several factors have been found to influence conductivity measurements. As leachate temperature increases, μAmp readings increase (Rowland and Gusta, 1977; McDonald and Wilson, 1979; Duke *et al.*, 1983; Bruggink *et al.*, 1991) and μmho readings increase (Tao, 1978). In addition, conductivity values are greater at extreme low temperatures due to the interference of normal membrane reorganization during imbibition (Tao, 1978). The source of water, distilled, deionized or tap water, in conductivity testing can be a source of error. Tap water has been determined to be unsuitable, distilled water sources vary and if not pure are also unsuitable; deionized water has been determined to be the best water source (Tao, 1978). Use of the same water source for each experiment is advised. Not only is the source of water important but it is suggested by Vosso (1984) to keep the ratio between the seed and the water volume in each cell (of the ASAC seed soaking tray) adjusted for seed size. Factors such as the length of imbibition period, initial moisture content, chemical treatment and variety dependent differences also affect electroconductivity values (Bruggink *et al.*, 1991). Damaged endosperm, pericarp (Wann, 1986 as well) and underlying tissues, caused higher leakage than damaged embryo (Bruggink *et al.*, 1991).

The seed coat, or pericarp in the case of maize, is the major controlling factor of the rate of water uptake and permeability (Woodstock, 1988). The testa forms a protective barrier against membrane damage caused by rapid water uptake

and electrolyte loss (Rowland and Gusta, 1977), thus a compromised testa results in higher conductivity values (Bruggink *et al.*, 1991). Pericarp for maize seeds has a similar function as the testa for other species. The testa has several functions: it serves to protect the underlying embryonic tissues, provides mechanical protection from injury, it physically holds seed parts together, it retains water and protects against desiccation following imbibition, controls the of rate of water uptake upon imbibition and protects from biotic stresses such as fungal attack (Woodstock, 1988). Leakage of electrolytes was much lower for soybean seeds with intact testae (Duke *et al.*, 1983). Though not specific about the type of damage, Tao (1978), Bondie *et al.* (1979) and Keys (1982) also found that mechanical damage increased conductivity values.

Low moisture soybean (*Glycine max*) seeds expressed a greater conductivity than high moisture seeds (McDonald and Wilson, 1979) apparently due to the rapid uptake of water and subsequent damage to membranes, sometimes causing the soybean seeds to crack (Tao, 1978). Rowland and Gusta (1977) concur that a more severe loss of cellular constituents occurs at lower moisture contents for faba beans (*Vicia faba*) and peas (*Pisum sativum*). The percentage of water within seeds before imbibition affects the rate of leakage, therefore conductivity values (Tao, 1978; Bruggink *et al.*, 1991).

The effect of seed size has been addressed in several publications. The results, however, have been inconclusive. For example, results from Hepburn *et al.* (1984) state that as seed mass increased so did the conductivity. Tao (1978) separated soybean seeds with five graduated sieves and McDonald and Wilson (1979) separated soybean seeds with two sizes of sieves; both studies found that

as the size of seeds increased so did the conductivity values. It seems likely that larger seeded cultivars inherently have higher conductivity values (Hepburn *et al.*, 1984; Siddique and Goodwin, 1985). McDonald and Wilson (1979) stated that accuracy in predicting germination decreased with increased seed size for two cultivars of soybeans yet no significant difference was observed in the standard germination test due to seed size. In fact, the predicted germinability of the large soybean seeds was underestimated each time. Matthews and Bradnock (1968) observed a decrease in correlation between emergence and conductivity when corrected for seed weight of French beans. It is likely that seed volume or surface area is a more appropriate correction factor than seed weight.

Another possible source of error when using the electroconductivity test is the difference in rate of water uptake of seeds, *i.e.* a large number of slowly imbibing seeds, or hard seeds in a sample would result in a deceptively low conductivity, which may not be a true indication of the seed quality and potential field emergence (Powell and Matthews, 1977). Ching and Schoolcraft (1968) reported that conductance measurements indicated that leaching or membrane degradation was a progressive process probably even continuing after death. The thickness and structure of the maize kernels were found to be related to the rate of moisture uptake and loss (Bruggink *et al.*, 1991).

Soak Time

Leakage begins when dry seeds begin to imbibe water, likely from the cytoplasm and vacuoles of cells (Simon and Raja Harun, 1972; Simon and Mills, 1983) or from the apoplastic regions (Kuo, 1986). It is unlikely that solute leakage originates from surface deposits on the seed since leakage continues even when seeds are transferred to fresh water (Simon and Mills, 1983). The time course of seed leakage is biphasic (Duke *et al.*, 1983; Simon and Mathavan, 1986; Kuo, 1986; Chuang and Sung, 1990) with an initially rapid phase lasting from 15 minutes to three hours, depending on the species, followed by a gradual prolonged slow rate. Simon and Mathavan (1986) discussed the idea that the time course of solute leakage remains the same for all species and differs only in the length of time involved, i.e. in celery (*Apium graveolens* L.) the initially rapid rate of leakage was confined to the first fifteen minutes whereas in flax (*Linum usitatissimum* L.) the initial phase lasted for about three hours. Interestingly, Simon and Mathavan (1986) noted that the total amount of leakage was generally greater for seeds which completed their initial rate very quickly, probably because they have a high surface/volume ratio, than for those with a prolonged biphasic pattern. Smaller seeds tend to have a high surface to volume ratio simply because of their geometry. Using a scanning electron microscope Chuang and Sung (1990) found that cracks formed in the seed coat during the first hour of imbibition for sorghum (*Sorghum bicolor* L. Moench) seeds and continued to develop over the next two hours.

The concentration of ionic material leached from the seed varies with the length of imbibition time[2]. The rate of ionic exchange varies throughout the soak

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[2] Neogen Food Tech. Corp., 620 Lesher Place, Lansing, Michigan, 48912, USA.

period but eventually a point is reached whereby the release of exudates from the seeds slows dramatically or even stops. It is the rate of leakage, not the total amount of leachable material, that is responsible for the differences which have been observed among cultivars in electroconductivity values (Rowland and Gusta, 1977). The rate of leakage during early imbibition may indicate membrane leakiness (Woodstock, 1988) since there is a difference in the rates of leakage with seed quality, i.e. high quality seeds leach material much more gradually than low quality seeds which leach material very quickly (Siddique and Goodwin, 1985).

Kuo (1986) and Brower and Mulder (1982) suggested reduced soak times of 6 hours for rice and 4 hours for beans, respectively, with the same prediction results as longer soak times. Additionally, long soak times introduce error into the system (Keys, 1982). Schmidt and Tracey (1989) found that discrimination between genotypes within each inbred background of maize could be established, and was stable, after just two hours of imbibition. However, the seed leachate conductivity values were not always significantly different until after four hours of imbibition. Total electrolyte leakage is reproducible after 150 minutes of imbibition in contrast to the extremely variable rates observed within the first 0 to 30 minutes of leakage (Duke *et al.*, 1983).

After an initially rapid influx of water with imbibition, the influx rate of water slows due to tissue hydration (Woodstock, 1988) stimulating the membranes to recover selective permeability (Simon and Raja Harun, 1972). It is only the outermost cell layers which are responsible for the rapid initial leakage of pea embryos (Simon and Raja Harun, 1972) and the substances located in the pericarp of maize are said to be responsible for the rapid initial rate of electrolyte leakage for

approximately the first hour (Bruggink *et al.*, 1991). Duke *et al.* (1983) indicated that the seed coat is a barrier to passive diffusion which is primarily responsible for electrolyte leakage from seeds during the initial phase. The release of seed leachate is the result of cell membrane degradation and the subsequent loss of control of permeability (Ching and Schoolcraft, 1968; Delouche and Baskin, 1973). Seeds in the dry state retain their lamellar configuration (Woodstock, 1988), subcellular structures and plasmodesmatal connections, but the cell walls are convoluted in characteristic patterns to which the protoplasm conforms (Webb and Arnott, 1982). It is this cytorrrhysis (cell wall folding) which preserves the structural integrity of seed tissue during dehydration (Webb and Arnott, 1982). Thus solute leakage may not be a result of membrane damage but of a temporary disorganization related to sudden rehydration (Bewley, 1986). It follows that passive diffusion is responsible for the first phase of leakage during imbibition (Duke *et al.*, 1983).

There is an increase in cell membrane permeability, or leakiness of the seeds, during imbibition associated with seed aging (Furman *et al.*, 1987). Water soluble ions and other electrolytes that leach from seeds can be quantified by determining the electrical conductivity of the leachate solution (Furman *et al.*, 1987). Seed quality measurements based on electroconductivity tests of individual seed leachates are a good indicator of plant performance relative to emergence rate and early plant growth (Mullet and Wilkinson, 1979). There is no easier or faster way to evaluate seed quality than with the conductivity method; the conductivity of 100 individual seeds allows for evaluation of the distribution of viability and vigor within a seed population.

Mathematical estimates or models

One approach for detecting seed lots with a low potential for emergence, or low vigor, has been the use of stress tests, another approach is to calculate an index to describe the pattern of germination over time, and in calculating any predictive index the aim is to try to summarize a set of data into a single statistic (Naylor, 1981). The conductivity method based on rates of electrolyte leakage is quick, easy, user independent, cheap and quantitative (Murray *et al.*, 1989). Steere et al. (1981) developed the method of germination prediction based on partitioning of μAmp data from the Neogen seed analyzer where an electronic count of seeds (100 seeds/sample) associated with μAmp values below the partitioning standard equates directly to percent viability. The partitioning value is species dependent and is determined by root mean square deviation, RMS (Freund, 1979), where G is the warm germination test result in percent (different for each species) and P is the predicted value from the analyzer for the same lot; N is the number of lots tested[3].

$$RMS = \frac{\sqrt{(G_1-P_1)^2 + (G_2-P_2)^2 + --- + (G_n-P_n)^2}}{N} \qquad (5)$$

A RMS represents the difference between standard germination test results and analyzer predictions for a group of seed lots, usually ten. It is the theoretical break point in current level that will divide the population into 'live' and 'dead' seeds

[3] Neogen Food Tech. Corp., 620 Lesher Place, Lansing, Michigan, 48912, USA.

(Bondie *et al.*, 1979). A RMS value is determined for each μAmp increment. The μAmp increment having the smallest RMS value is considered the partitioning standard for the species. The smaller a RMS the better is the correlation between warm germination and analyzer test results. The manufacturer suggests soak times of 18-24 hours for hybrid maize, 48 hours for alfalfa, red clover, carrot, onion, radish, sunflower and 72 hours for cabbage. The methodology is deficient in two fundamental principles; RMS has no biological rational and the manufacturer's suggested soak times would likely be injurious if tested seeds are to be preserved. Wilson (1992) corroborated the inappropriateness of the partition method; furthermore, he found that this method showed poor accuracy when used to predict germinability.

The partition value is determined empirically through many studies and the ASAC manual recommends the use of samples which represent extremes in quality, i.e. dead seeds and very high quality seeds. Such extreme lots of seed are difficult to locate since most companies discard their dead seeds. The partition setting is a value which is selected by the laboratory technician who is performing an experiment and, therefore, could be dependent on that person's ability to perform the proper calculations from day to day without subjective influence, leaving room for human error. Another source of error arises from the use of one cultivar to calculate the partition value, since this value cannot be considered as an absolute boundary between viable and non-viable seeds due to the overlap in conductivity values which occurs between these two categories. A single partition value for the species will ultimately lead to underestimation or overestimation of germination depending on which variety is used to calculate the partition (Hepburn *et al.*, 1984).

The relationship between viability and electrolyte leakage has been demonstrated previously; lower conductivity values are associated with higher quality seed (Parrish and Leopold, 1978; Keys, 1982). Histograms, or population distribution graphs, of current values (μAmps) are good indicators of seed lot quality (Bondie *et al.*, 1979). Moore *et al.* (1988) suggested that the idea of shape of the cumulative frequency distribution be retained when estimating seed quality in an article that discusses the use of the flexible Richards function to fit single seed conductivity data. The Richards function is a non-linear, flexible, asymptotic function and as such will fit both normally and non-normally distributed data (Causton *et al.*, 1978; Moore *et al.*, 1988; Nath *et al.*, 1990). Current value (μAmps) distributions are non-normal (Wilson, 1992). Internal slope is derived from four Richards function parameters using a special purpose program (Nath *et al.*, 1990). A unique and important advantage of the program is that initial parameter values are not required, it initializes its own, whereas non-linear models do not.

Internal slope is a non-central tendency measure of viability which may be defined as the maximum slope of a cumulative frequency distribution (CFD) of individual seed leachate conductivity values where the sample is 100 seeds. Internal slope essentially reflects the shape of the CFD of μAmps; it is determined as the derivative of the curve fitting function at the inflection point of the curve where cumulative frequency is a function of cumulative μAmps given a total frequency of 100 seeds. The CFD is sigmoidal and it has a relatively constant slope between inflections which can be approximated by a line tangent to the inflection point of the curve symbolized by $(dY/dX)_{MAX}$ (Moore *et al.*, 1988). The Richards function has historically been used to model single leaf, whole plant, fruit and root

development, where time represents the independent variable (Garrett *et al.*, 1989). The fitting is carried out in two stages: first the starting values are determined and second the constants *A, b, k* and *N* are estimated using Newton-Raphson iterative method (Garrett *et al.*, 1989; Nath *et al.*, 1990; Nath *et al.*, 1993). The Richards function, equation **(6)**, is a generalization of the logistic function and as such fits data sets that exhibit a limit to growth, i.e. an asymptote.

$$\log_e W = \log_e A - \left(\frac{1}{N}\right)\log_e(1 \pm \exp(b - kt)) \qquad \textbf{(6)}$$

It was first proposed for use in plant growth analysis by F. J. Richards in 1959 and later by Causton *et al.*, in 1978.

A steep internal slope was observed for high quality lettuce (*Lactuca sativa* L.) seeds (Moore *et al.*, 1988). The greater the internal slope value the less seed-to-seed variation and the higher the seed viability (Nath *et al.*, 1990). Viability decrease is associated with an increase in solute leakage and a decrease in internal slope. Internal slope was developed to monitor seed deterioration and estimate seed viability (Moore *et al.*, 1988).

Arithmetic means, medians and modes of frequency distributions are generally used to describe populations and may qualify as indicators of seed quality, but means are strongly affected by extreme values and all three values are measures of distribution location whereas internal slope is not associated with distribution location, giving it wider application (Moore *et al.*, 1988). Poor quality seeds exhibit not only low germination but sporadic germination relative to time of germination. Internal slope summarizes this information, i.e. the larger the internal

slope value the less variation among quality of individual seed and the higher the quality. The reciprocal of internal slope is also a measure of seed to seed variability in leakage. Internal slope could provide a description of the state of deterioration of a seed sample and assist in development of non-destructive methods for seed viability determination. The focus on shape rather than distribution location parameters acknowledges that different sub-populations within a species may reveal a different relationship between leachate conductivity and seed performance. Wilson (1992) suggested that this method neglects the underlying biology that leaky seeds are bad; however, this method accounts for the shape of the frequency distribution which includes all of the seeds in the sample, good or bad.

It is important to evaluate seed vigor from frequency distribution histograms or from population statistics that describe the shape of the distribution and/or from indices created from these population statistics which may take the mean value and standard deviation into account. Frequency distributions become broader, decrease in peak height and generally shift to higher values with increasing deterioration (Furman *et al.*, 1987; McDonald and Wilson, 1980). Skewness and kurtosis are two such indices suggested by Furman *et al.* (1987) since they describe the shape of the frequency distribution and do not depend on the magnitude of the readings. Skewness is a quantitative value of the departure of a distribution from symmetry and kurtosis is a quantitative value that expresses the relative peakedness of a distribution.

Wilson (1992) described how the mean current value, μAmps, of 100 individual seed leachate values resembles the bulk conductivity method and suggested using the median which is not as severely affected by extreme values or

skewness. Wilson (1992) suggested that the mean current value, μAmps, best predicted field emergence, although the median achieved almost the same results. Linear regression on the μA mean provided as good a prediction of germination percentage as use of an optimized partition.

Curve fitting of leakage data has been done by Murray *et al.* (1989) for quantifying frost damage in red spruce [*Pica rubens* Sarg. syn. *P. rubra* (Du Roi) Link]. They fit the first order equation

$$C_t - C_o = (C_{auto} - C_o)(1 - e^{-kt}) \qquad (7)$$

to bulk conductivity data and found that they could predict shoot death following freezing treatment before the appearance of visible symptoms. Equation **(7)** does not constrain the curve to pass through the origin, however. Thus any non-zero initial conductivity is underestimated while the initial slope of the curve is overestimated by the equation. It has been demonstrated that seed leakage is biphasic (Simon and Mathavan, 1986), therefore it is non-linear, but straight line equations are the easiest of all to manage (Gold, 1977). After much searching for a non-linear model, the rectangular hyperbola (Bliss and James, 1966), which is asymptotic to two axes, and the monomolecular function seemed to be the two best possible functions to fit seed electrolyte leakage data, i.e. cumulative μAmps versus soak time.

The monomolecular equation was considered as a model for seed leakage as a function of time because it is non-linear, asymptotic and does not have an inflection. It describes the progress of a simple irreversible first-order chemical

reaction (France and Thornley, 1984) and has certain assumptions associated with it; i.e. the quantity of growth machinery is constant and independent of dry weight, *W*; this machinery works at a rate proportional to the substrate level, *S*; and growth is irreversible. The equation is

$$W=W_f(1-e^{-kt}) \qquad (8)$$

where $W_f - W = S$, k is a constant and t is time. The monomolecular function has no inflection point and does not necessarily have to have a lower, or second, asymptote (Causton and Venus, 1981) as does the rectangular hyperbola. Johnson (1987) used a two parameter form of the rectangular hyperbolic function to model the efficiency of the growth process of a plant as a function of the specific growth rate which may be rewritten as

$$\mu Amps=\frac{At}{Am+t} \qquad (9)$$

so that A represents the asymptotic maximum leaching in terms of μAmps, t is soak time and $1/m$ represents the inital slope, also the maximum slope. The rectangular hyperbola approaches its asymptote very slowly when compared to the monomolecular function (Causton and Dale, 1990); nevertheless this is how seeds leach electrolytes during the testing process. This model is appropriate when the asymptote and the m parameter are constant (Johnson, 1987). There is little biological rationale for using the monomolecular equation with seed leachate conductivity data, whereas there is much biological rational for using the

rectangular hyperbola, namely its current use in enzyme kinetics (Thornley and Johnson, 1990; Ratkowsky, 1990). Considering seed leachate conductivity, as soaking continues, conductivity values approach an upper asymptote. The two parameter rectangular hyperbolic function can be linearized (Bliss and James, 1966; Gold, 1977; Johnson, 1987) as follows

$$\frac{1}{\mu Amps} = \frac{1}{A} + \frac{m}{t} \qquad \textbf{(10)}$$

Therefore it is intrinsically linear. However, with computer software packages which model non-linear regressions without any trouble at all, it isn't necessary to transform the primary data to the linear form. Also, when linear transformations of the rectangular hyperbola are done utilizing equation **(10)**, one must remember that the error associated with values of t are transformed as well; thus the linear fit is usually distorted (McIntosh and McIntosh, 1980; Johnson, 1987). The rectangular hyperbola has been named the single most useful equation available to the plant modeler (Thornley and Johnson, 1990).

A problem associated with the rectangular hyperbola, as discussed by Ratkowsky (1990) is that the parameter estimates are far-from-linear in their estimation behavior, consequently the estimates of the parameters are biased and the confidence intervals for these parameters, based on the usual linearization, method are inaccurate. Computation of likelihood, or lack-of-fit confidence limits, provides a much better method for non-linear regression parameter estimation. Lastly, the degree of non-linear behavior tends to increase with the number of parameters.

Seed Aging

Aging is deterioration through time. The three main factors associated with seed deterioration are time, temperature and moisture content (Ellis and Roberts, 1980) or relative humidity of the storage atmosphere (Priestley, 1986). Seed deterioration is progressive and although the loss of germination is the final manifestation of deterioration, stability of germination percentage during storage does not mean that deleterious changes have not already occurred (Gill and Delouche, 1973). The factors involved in seed deterioration led Ellis and Roberts (1980) to develop their mathematical model to predict seed longevity.

Many tests have been developed to predict the relative storage potential of seed lots including the rate of respiration during the early stages of imbibition and germination, seedling growth rate, cold tests, glutamic acid decarboxylase activity, electrical resistance of seed leachates and tetrazolium reactions (Delouche and Baskin, 1973). Accelerated aging was initially developed to evaluate the relative storage potential of seeds (Delouche and Baskin, 1973) and is currently used to study seed deterioration (Nath *et al.*, 1991). Accelerated aging for seed vigor testing is rapid, inexpensive, simple, universal for all seeds, has the capability for individual seed evaluation and requires no additional training for seedling evaluation (McDonald, 1977). Roberts (1981) stated that the standard germination test does not have sufficiently small confidence limits to detect important differences in seed quality at high viability levels and an accelerated aging test can pick up these differences more easily. Delouche and Baskin (1973) discussed the importance of being able to determine which seed lots are suitable for storage. There are serious implications when an apparently good quality seed lot fails to maintain germinability

during storage (Delouche and Baskin, 1973). The standard germination test is not reliable as the sole measure of physiological quality and storage potential as it has many deficiencies such that many seed lots of a certain species with comparable initial germination percentages proved to have greatly varying storability potential (Delouche and Baskin, 1973; Roberts, 1981).

Physiology of Seed Aging

Aging methods have provided the experimental material to study the elusive aging process. Many papers have been published in the last decade on the physiology involved in the process of aging (Ghosh, Adhikary and Banjeree, 1981; Roberts, 1981; Perl *et al.*, 1987; Ganguli and Sen-Mandi, 1990; Basavarajappa *et al.*, 1991) which involve deterioration of many systems within the tissues (Roberts, 1981). Electroconductivity of seed leachates increases with the duration of aging (Ching and Schoolcraft, 1968; Gill and Delouche, 1973; Harrington, 1973; Bondie *et al.*, 1979; McDonald and Wilson, 1980; Ghosh *et al.*, 1981; Steere *et al.*, 1981; Keys, 1982; Saxena *et al.*, 1985; Powell, 1986; Furman, *et al.*, 1987; Pandey, 1988; Bruggink *et al.*, 1991; Nath *et al.*, 1991). Powell and Matthews (1977) found an increase in conductivity even when there had not been a decline in viability. Bruggink *et al.* (1991) found that with an increase in aging there was increased leakage of solutes from embryonic tissue. Ghosh *et al.* (1981) found an increased amount of free amino acids and sugars in the leachate with aging; additionally, aging embryos showed a decreased ability to incorporate ^{3}H leucine into protein, ^{14}C uridine synthesis into RNA and low incorporation of $^{32}PO_4$ (in intact as well as excised embryos). Levels of RNA and inorganic phosphate increased, as

did the leaching of proteins and sugars, as aging progressed (Saxena *et al.*, 1985). Initial differences in quality between seed lots are the result of differences in genotype and differences in their pre-storage history. With time, tissues become more leaky, implicating membrane damage, dehydrogenases become less active and the ability to incorporate leucine, uracil and phosphate into metabolites declines (Roberts, 1981). Seedlings exhibiting these symptoms are less vigorous, the seedling growth rate is reduced and a greater number of abnormal seedlings are present (Roberts, 1981). With aging there is a loss of activity of many enzymes, more permeable membranes and progressively more abnormal seedling growth leading to seed death caused by the accumulation of toxic materials, the destruction of triggering enzymes, such as gibberellic acid and cytokinins, or the mechanism of their production or by denaturation of the histone, enzymatic and membrane proteins (Harrington, 1973).

As moisture content of air dry seeds, stored at 30°C, was increased the viability period was greatly decreased and the number of abnormal seedlings was greatly increased (Villiers and Edgcumbe, 1975). Ching and Schoolcraft (1968) studied the physiological and chemical differences in aged crimson clover and rye seeds and found that with increasing seed moisture and storage temperature there was (a) a decrease in the starch and sugar content; (b) an increased amount of free amino acids; (c) active protease in stored seeds related to hydration and temperature; and (d) an increase in inorganic phosphate for crimson clover, but not for ryegrass, which probably originates from phytin via the activity of phytase. These findings implicate the activity of both proteases and phytases in the aging process. Proteases degrade vital structural and insoluble proteins of organellar

membranes, nucleoproteins, ribosomes and enzymes. Phytases hydrolyze phytin, the most abundant source of inorganic phosphate located in the aleurone layer of the endosperm, to release phosphate, cations and myo-inositol (Bewley and Black, 1985). Myo-inositol is subsequently used for the synthesis of cell walls during seedling growth. Activity of proteases and phytases increase after germination (Bewley and Black, 1985).

Ganguli and Sen-Mandi (1990) suggested that non-germination of intact seeds is the result of the loss of amylolytic activity. When sucrose was supplemented to the growth medium, the percentage germination for both naturally and artificially aged seeds was improved. This suggested to Ganguli and Sen-Mandi (1990) that sucrose fulfilled the requirement for utilizable substrate which had become limiting in the aged seeds due to the loss of amylolytic activity. Ungerminated cereal grains contain little or no α-amylase but upon germination there is a dramatic increase in the synthesis of α-amylase in the aleurone layer of cells which surround the endosperm (Goodwin and Mercer, 1983). Livesley and Bray (1991) set out to determine the effects of aging on the loss of viability with respect to the aleurone layer which adheres to the inside of the testa of the wheat seed. The integrity of the aleurone layer was measured by its ability to synthesize and secrete α-amylase during germination. Aging consisted of ambient storage from the date of harvest in 1986. They found that in seeds which germinated normally, total α-amylase activity increased between days three and six after germination. However, no increase was observed for abnormally germinating seeds, suggesting that the ability of the aleurone tissue to respond to the appropriate stimulus had been lost.

Amylolytic and proteolytic activity increases during and after germination (Berrie and Drennan, 1971; Goodwin and Mercer, 1983). Acid soluble proteins (histone) were found to increase similarly for both naturally and artificially aged seeds, presumably to assist in maintaining DNA integrity in aging embryos thus enabling them to germinate after completion of any necessary DNA repair (Ganguli and Sen-Mandi, 1990). However, Livesley and Bray (1991) observed that ^{14}C labelled amino acids in the aleurone layer were incorporated in the abnormally germinating seeds, suggesting no obvious defect in protein synthesis. The abnormally germinating state may represent the stage during seed aging immediately prior to the total loss of viability of seeds.

A popular theory to explain membrane deterioration is that the aging of seeds is caused by lipid peroxidation, which is responsible for membrane perturbation consequent to its alterations of unsaturated fatty acids (Priestley *et al.*, 1980), i.e. aging is at least in part due to autooxidation of unsaturated fatty acids by free-radicle formation. The theory of lipid oxidation of unsaturated fatty acids leading to the formation of free radicals which then damage cellular membranes and react destructively is recognized by many researchers (Harrington, 1973; Pammenter *et al.*, 1974; Villiers and Edgcumbe, 1975; Harman and Mattick, 1976; Priestley *et al.*, 1980; Perl *et al.*, 1987; Lin and Pearce, 1990; Basavarajappa, *et al.*, 1991). The theory of the mechanisms of lipid oxidation indicate that fatty acids with two or more unsaturated bonds should be more labile and prone to form free radicals than more highly saturated acids. Thus, if fatty acid oxidation and free radical formation are occurring, the highly unsaturated acids would decrease as seeds deteriorate, whereas the quantity of saturated fats would remain constant

(Harman and Mattick, 1976). Powell (1986) separated this phenomenon into two stages: first there is formation of free radicals by peroxidation and second there are changes in the amount of membrane phospholipids due to hydrolysis. Harman and Mattick (1976) found decreased viability with aging pea seeds where the concentrations of the most commonly found saturated fatty acids, palmitic (16:0), stearic (18:0) and oleic (18:1), did not change with aging or fungal infection in either the whole seed or the embryonic axes. The concentrations of the dienoic and trienoic acids, linoleic (18:2) and linolenic (18:3), were affected by both storage and infection. Linoleic acid decreased with both storage and infection in whole seeds while no significant change was observed in the axes. There was a more rapid decrease observed for linolenic acid in the whole seeds as well as for the axes, which paralleled the loss of vigor of the seeds. This suggests that free radical formation may be a factor in the loss of seed vigor and, ultimately, seed death.

Basavarajappa *et al.* (1991) did an intensive study of various biochemical changes associated with aging of maize seeds and found that (a) there was a decrease in total phospholipid with the aged seeds; (b) there was an increase in absorbance indicative of triene and tetraene formation for the single cross seeds but only triene formation in the double top cross; (c) malonaldehyde content (a product of lipid peroxidation) increased with aging and was probably associated with unsaturated fatty acids and membrane damage; (d) a significant increase of total free fatty acids was possibly due to the catalytic activity of esterolytic enzymes; (e) phospholipase A activity increased indicating the loss of membrane phospholipid and subsequent lipid peroxidation; (f) the peroxidation activity decreased with aging

possibly making the seed more susceptible to the effects of oxygen and free radicals on membrane unsaturated fatty acids and to the production of secondary lipid peroxidation products such as malonaldehyde and lipid conjugants; (g) there was a loss of ascorbate with aging further supporting the possible lipid peroxidation and increase in membrane lability; (h) the content of total carbohydrate and reducing sugar declined during aging which could be due to their respiratory utilization for energy or due to an increase in amylase activity; (i) there was a 50% decrease in water soluble protein content at the end of the aging regime which could be related to the denaturation of protein during artificial aging; (j) there was a substantial increase in total free amino acids after aging, possibly due to hydrolysis of protein which was further supported by high protease activity detected after aging; (k) there was a decrease in the activities of acid phosphatase and phosphomonoesterase after aging affecting the phosphate metabolism in the seeds which in turn would affect the adenylate energy change, which relates the adenine nucleotides and their ratios, key factors in metabolic regulation; and (l) there was a gradual decrease in the activity of dehydrogenase with aging which may be associated with their low levels of ATP production and reduced rate of ATP and GTP dependence on protein synthesis. These data provide clear evidence for the loss of membrane integrity as the probable first deteriorative change during aging maize seeds. The mechanism proposed by Basavarajappa *et al.* (1991) involved in seed deterioration is as follows: the free radicals produced as a result of lipid peroxidation due to aging react with the membrane lipids and ultimately destroy the structure of cellular membranes and denature the food reserves. Saxena *et al.* (1985) found that for sesame (*Sesamum indicum* L. Cv. Gujarat-1) seeds that had

undergone accelerated aging not only did viability and vigor decline, but so did catalase, peroxidase and total dehydrogenase activity in parallel with percent germination. Furthermore, activities of hydrolytic enzymes such as invertase, RNase and acid phosphatase increased with aging in dry seeds, but during germination activity of these enzymes exhibited decreases with aging.

Lin and Pearce (1990) used 'ambient' conditions, 79% relative humidity and 25°C, as well as moderate, 80% relative humidity and 40°C, and severe, 100% relative humidity and 45°C, accelerated aging conditions for french bean (*Phaseolus vulgaris* L. cv. The Prince) and sweet corn (*Zea mays* L. cv. F1 hybrid First of All) in their study of lipids. They found that (a) phosphatidic acid was not present in maize seeds and that the content of phosphatidylcholine was not significantly lower at the end of aging; (b) fatty acid composition of total lipids present did not change with aging; and (c) only with the severe aging treatment did total phospholipid contents differ significantly between the beginning and the end of aging. In general they found no obvious relationship for maize seed aging, germination or vigor loss and lipid composition. On the other hand, french bean results showed that hydrolysis of phospholipids occurred during all three aging treatments and resulted in the depletion of total phospholipid and conversion of a substantial proportion to phosphatidic acid. The relative content of different fatty acids changed markedly with aging, the general trend being for percent linolenic acid (18:3) to fall, then rise, while palmitic acid was unchanged and the content of oleic (18:1) and linoleic acids (18:2) changed in the opposite direction to linolenic acid. This early fall in linoleic acid is consistent with peroxidation being an early event in aging, its later fall possibly coincides with the time when percent germination reached zero. Lipid

changes seem unimportant in aging of maize under a wide range of conditions and either lipid hydrolysis and peroxidation (before germination) do not play a role in extensive membrane disorganization or undetected, possibly minor, lipid changes can have considerable structural consequences (Lin and Pearce, 1990).

Absence of a demonstrable alteration of the unsaturated fatty acids indicates that membrane-related or other effects of aging may not be mediated through lipid peroxidation for soybean and maize (Priestley *et al.*, 1980). This led Priestley *et al.* (1980) to study the changes in tocopherols, which act as natural antioxidants and are associated with both membrane and storage lipids and serve to prevent the onset of free radical formation (Bewley, 1986); analysis of organic free radical status in naturally and artificially aged seeds was done using Electron Spin Resonance (ESR). None of the tocopherol contents showed a relationship with seed age or percent germination, and the organic free radicals present in the cotyledons of naturally and artificially aged seeds showed little change with age. The constant nature of the ESR signal in soybeans, coupled with the absence of marked change in the tocopherol fraction, led Priestley *et al.* (1980) to the conclusion that lipid peroxidation need not be a corollary of natural or artificial seed aging. It was surmised that lipid peroxidation may become significant in soybean seeds *post mortem*.

Methods of Aging Seeds

Many methods have been developed for artificially aging seeds (Delouche and Baskin, 1973; Tao, 1979; Matthews, 1980; Bhattacharyya, 1985; Bruggink, 1989; Vertucci and Roos, 1990). Delouche and Baskin (1973) developed what is

known as accelerated aging. This technique has been used for a wide range of products such as insulation on electrical wiring and tent canvas (Delouche and Baskin, 1973). Tao (1979) used a modified version of accelerated aging by putting seed samples into sealed jars. Matthews (1980) developed the controlled deterioration method of artificially aging seeds. Bruggink (1989) investigated an alternative to the controlled deterioration method of artificially aging seeds. Vertucci and Roos (1990) initiated an entirely new approach to artificially aging seeds utilizing a very dry atmosphere and high temperature.

The technique used by Delouche and Baskin (1973) involves the exposure of small samples of seeds from many seed lots of the same species to very adverse conditions such as 100% relative humidity and temperatures of 40°C to 45°C, for a specific period, ranging from two to eight days, then determining the survival by standard germination tests. The temperature and length of aging is dependent upon the species used. Occasionally a less severe aging regime of 30°C and 75% relative humidity with exposure periods of 6 to 24 weeks was used and yielded better results. Varying levels of deterioration of different high quality seed lots is the underlying theory behind the accelerated aging process as proposed by Delouche and Baskin (1973) in that the loss of storage potential and incidence of seedling abnormalities increase as the germination rate decreases with increased seed deterioration. Ching and Schoolcraft (1968) found that even at low temperatures, 3°C, crimson clover and ryegrass seeds lost all viability when stored at high moisture contents, ranging from 12 to 20%.

However, the storage discussed by Delouche and Baskin (1973) is open storage in a warehouse in Mississippi and not the ideal storage conditions exercised

at germplasm storage facilities. Deterioration within a population of seeds is on an individual seed basis, resulting in a diversity of final response. Thus the degree of deterioration of that population ranges from relatively non-deteriorated to non-germinable (Delouche and Baskin, 1973). The physiological symptoms of deterioration include the loss of enzyme activity, reduced respiration, increase in seed leachates and increase in free fatty acid content (Copeland, 1976). Mitochondrial structures become distorted and disorganized, and there is reduced ATP production and efficiency, failure to synthesize proteins upon imbibition accompanied by loss of RNA synthesis and processing (Bewley and Black, 1985). Also, an increase in chromosome damage has been suggested (Roos, 1984; Bewley and Black, 1985). Powell (1986) considered many of these symptoms as imbibitional damage caused by rapid water uptake. The loss of membrane integrity has been implicated by numerous researchers as the result of deterioration in seeds (Priestley, 1980; Ghosh *et al.*, 1981; Roberts, 1981; Bewley and Black, 1985; Saxena *et al.*, 1985; Perl *et al.*, 1987; Basavarajappa, 1991). All of these considerations are part of a seed lot's pre-storage history which may be caused by weathering, time of harvest, harvesting procedure, drying, handling and conveying, processing and treating (Delouche and Baskin, 1973); thus different seed lots have different pre-storage histories and different physiological conditions, or levels of deterioration. This is the inception of the inadequacies of the germination test (Gill and Delouche, 1973). Variability exists among seeds of any given seed lot for dimensions and mass, plus each seed is affected independently by environmental conditions during development (Dornbos and Mullen, 1987). Delouche and Baskin (1973) found that the response to high humidity and temperature conditions over

days and weeks was indicative of a seed lot's storability over months and years in a non-conditioned, or open, warehouse in Mississippi. The seed lots which maintained well during accelerated aging, stored well whereas those that were substantially reduced in germination stored poorly; there were few exceptions. Reproducibility of this method is dependent upon precisely controlled conditions (i.e. temperature variation should not exceed ± 0.5°C).

Delouche and Baskin assume that the processes of deterioration under accelerated aging conditions are similar to those under 'normal' conditions except that the rate is greatly increased. Roos (1989) cited three reasons why this is not the case: (1) during high temperature and high moisture conditions seed moisture is elevated to such an extent that microorganisms can easily grow; (2) there appears to be a stage of deterioration that occurs with slow or natural aging that is eliminated by rapid aging, namely an increase in the number of abnormal seedlings concomitant with decreased percentage germination; and (3) a lower percentage of chromosomal aberrations has been observed for rapid aging than for slower aging. It is possible that the accelerated aging of seeds does not speed up the aging process; instead, the loss of germinability is the result of compounding effects of aging with imbibitional stresses (Priestley, 1986). Ganguli and Sen-Mandi(1990) concluded that the two conditions that cause deterioration, natural or artificial aging, may bring about completely different effects in cellular events of the embryonic axis and that apparent similarity in germination performance might not necessarily indicate similarity in the inherent ability of deteriorating seed lots. Nevertheless, artificial methods of aging seeds remain necessary when naturally aged seed lots with various germination responses are unavailable.

Tao (1979) compared three accelerated aging methods for soybeans, the large chamber method, the sealed jar method and a modified tray method. He reported that with the large chamber method, like that of Delouche and Baskin, there were significant variations in accelerated aging test results, namely final seed moisture content, fungal growth and percent of high vigor seedlings among samples from the same seed lot, depending upon their location in the accelerated aging chamber. Most of these differences were attributed to condensation formation and subsequent dripping onto the seeds. With the sealed jar method, Tao reported consistent results; furthermore he was able to confirm that condensation had occurred in the large chamber, but because the jars themselves were sealed the seeds inside were not affected. He proposed that seeds with higher initial moisture contents had a lower percentage of high vigor seedlings but found that there was no significant difference in germination after three days of aging between seed samples with moisture contents of 13.0 and 8.8% (both of which would routinely occur in a seed testing laboratory). It has been recognized that initial seed moisture influences the rate of accelerated aging (McDonald, 1977; Tao, 1979; Roos, 1984) *i.e.* high moisture seeds deteriorate more rapidly than low moisture seeds resulting in more injurious seedling growth responses during germination. An important fact that should not be overlooked is that seed lots with low seed vigor tend to deteriorate much more rapidly than those with high vigor (Roos, 1984).

Matthews (1980) discovered a problem associated with the accelerated aging methods used by Delouche and Baskin (1973) and Tao (1979). The different seed lots had different rates of moisture gain from the humid atmosphere, which meant that differences found in response to aging resulted not only from pre-

storage history but also from how quickly they reached an elevated moisture content. Matthews improved on the accelerated aging method with his controlled deterioration method, which involves placing seeds of a known moisture content onto a moist blotter paper and allowing the seeds to imbibe to the required moisture content, depending upon the species, which is checked by frequent weighing. The partially imbibed seeds are sealed in a container and held overnight at 10°C to ensure an even distribution of moisture, then sealed into an aluminum foil seed packet which is incubated in a water bath at 45°C for one day, after which time the seeds are placed on a moist blotter paper at 20°C to germinate. Matthews did find that germination after controlled deterioration was more closely related to relative field emergence than standard germination which attempts to achieve the highest possible germination rate under the best conditions. Controlled deterioration, however, was not effective at predicting comparative emergence for every crop in the two years the study was conducted. Matthews also pointed out that there were many abnormal seedlings as a result of the deterioration treatment and so he simply counted the emergence of the radicle as an indication of germination rather than counting abnormally germinating seedlings as dead. Another method was developed by Bhattacharyya (1985) whereby the seed samples were wrapped in cheesecloth and submerged in hot water, 58°C ± 1°C for varying lengths of time. A problem with these two methods is that the seeds directly contact water resulting in solute leakage. Bruggink (1989) developed an improved controlled deterioration method whereby the seeds are equilibrated with a suitable relative humidity to promote more uniform uptake of water. After maize seeds were held over a saturated solution of sodium chloride, relative humidity of 75% (O'Brien, 1948), for

two weeks they were sealed in aluminum foil seed packets and heated in an oven. Greater relative humidities produce very moldy seeds (Bruggink, 1989; Roos, 1984). Bruggink's procedure did not involve direct contact with water, avoiding solute leakage, and controlled moisture contents were maintained.

An entirely different approach by Vertucci and Roos (1990) employs the idea of accelerating the seed aging process in a hot and dry environment, specifically 35°C and 0% to 40% relative humidity. This was attempted to understand the comparison of measurements of physiological activity, specifically measurements of lipid content, the physical status of water using moisture sorption isotherms, oxygen uptake and storage (for two to nine months) with the increased viscosity in the aqueous domain of seeds. These conditions may lead to a form of artificial aging. An objective of this type of aging would be to achieve a slower rate of deterioration to more closely mimic natural seed deterioration and aging. The end result of extreme drying and of high moisture contents are the same, a loss of viability (Nutile, 1964).

A dry seed is a viscous system whereby the rate of chemical reactions can be greatly influenced by the intermolecular binding energy of water (Vertucci and Roos, 1990). There are three regions of water binding: region 1 is the water which is very tightly bound to ionic groups, it is considered 'structural water'; region 2 is water that is weakly bound to polar, nonionic groups; and region 3 is water which is very weakly arrayed as bridges over hydrophobic moieties and resembles bulk water (Vertucci and Leopold, 1987; Vertucci, 1989). Dehydration increases the viscosity and dramatically reduces reaction rates in aqueous solutions to negligible levels (Vertucci and Roos, 1990). When the removal of water in region 1, of

soybean and pea seeds, occurred there was a progressive loss of viability observed (Vertucci and Leopold, 1987). Furthermore, reducing the water content in seeds does not prevent physiological and injurious chemical activity because oxidative activity which is attributed to lipid peroxidation occurs at low moisture contents (Villiers and Edgcumbe, 1975); oxidative activity is not attributed to the mitochondrial electron transport (Vertucci, 1989; Vertucci and Roos, 1990) or glycolysis (Vertucci, 1989) at low moisture levels. The drier the seed (below moisture contents of 4 to 6%), the greater the amount of lipid autooxidation (Harrington, 1973; Vertucci, 1989). Vertucci and Roos (1990) observed a reduction in germination for seeds stored at 1 to 8% or greater than 40% relative humidity within six to nine months for sunflower (*Helianthus annus*), peanut (*Arachis hypogea*), lettuce, soybean and pea. It was suggested by Vertucci and Roos (1990) that very low moisture contents when compared to high moisture contents have similar deleterious effects to seed viability because when the water which is tightly associated with macromolecular surfaces, providing protective effects, is removed, deterioration of those macromolecules ensues. Furthermore, they suggested that there is an interaction between the properties of water in an unimbibed seed and the types of reactions that can occur.

Powell and Matthews (1977) contend that dry storage, 1% relative humidity and 10°C, of pea seeds caused little change in viability or leachate conductivity. Likewise, Ellis *et al.* (1990) reported that desiccation injury is rare in orthodox seeds. Lowering the moisture contents of seeds in air dry storage is expected to increase longevity (Villiers and Edgcumbe, 1975). It has been suggested that ultra-dry storage of seeds (i.e. storage of less that 5% moisture content) could be a

promising technique and might permit refrigeration costs to be reduced and possibly avoided in some applications (Ellis *et al.*, 1990). Ellis *et al.* (1990) calculated that, by decreasing seed moisture content in storage from 5.0% to 3.5% for onion and 2.0% for peanut, longevity could be increased by factors of 8 and 42, respectively. Therefore, it is worth pursuing ultra-dry storage of oily seeds, such as those mentioned, since they store poorly at higher moisture contents (which are still considered low for starchy seeds). Removal of the weakly bound water fraction within seeds increases longevity. The strongly bound water fraction begins to be removed at water potentials below -350 MPa and this water has little or no further influence on longevity presumably because it has little chemical potential (Ellis *et al.*, 1990).

Soaking Injury

The loss of essential material from the seed, the greatest proportion of which are leached out in the first phase of imbibition, is one of the most probable causes of soaking injury (Saxena *et al.*, 1985; Weges and Karssen, 1990). Roos and Pollock (1971) proposed that soaking injury is caused by the absorption of water, during imbibition, which then obstructs the apoplastic regions (intercellular spaces) and impedes oxygen diffusion into the cells, which is necessary for stimulating growth. Roberts (1981) pointed out that the rate of aging in seeds is not only dependent upon moisture content and temperature but also on oxygen pressure. Seeds in the dry state do not experience normal metabolism, therefore sub-cellular repair and turnover mechanisms remain inactive (Villiers and Edgcumbe, 1975; Roberts, 1981).

Intermittent wetting interspersed within dry storage is sufficient to keep viability high and avoid the accumulation of chromosomal aberrations (Villiers and Edgcumbe, 1975). Nath *et al.* (1991) found no significant effect for unaged seeds which had undergone a short hydration then dehydration treatment, and that a long soak period, i.e. 24 hours, caused a great reduction in viability, suggesting that a two hour soak offers protection against deterioration in storage whereas long soak periods increase the rate of deterioration in storage despite improved germinability. Berrie and Drennan (1971) also suggested that short soak periods were protective and long soak periods increased the rate of deterioration. Seeds may be primed for increased vigor by imbibing then drying back (Woodstock, 1988). Hydration-

dehydration treatments produce greater root and shoot lengths than untreated seeds, this could be the result of repair mechanisms operating during the first phase of soaking and when stopped by dehydration results in the cessation of the third phase, germination, before causing embryo damage (Pammenter, 1974; Villiers and Edgcumbe, 1975; Younis *et al.*, 1991). Studies such as these indicate that the change in membrane systems is not one of degradation of membrane constituents on drying with resynthesis on imbibition, rather it is a physical change whereby the membranes can be switched from leaky to intact with ease and rapidity (Simon and Raja Harun, 1972). Repair and replacement mechanisms are most likely activated during this first phase of hydration just prior to synthesis of proteins and RNA along with food reserves being mobilized, and this first phase may be stopped at any point by dehydration without causing embryo damage. These metabolic changes are retained in the seed upon drying, reducing the lag phase of germination when resupplied with water (Savino *et al.*, 1979). As long as the seeds remain intact, membranes seem to be desiccation tolerant. Savino *et al.* (1979) found that treated (soaked) seeds maintained viability and vigor for an extended period compared to untreated controls.

Models and Responses

Interaction of two factors may be explained by selecting a 'best subset' model, a polynomial selected from a full or complete model, and constructing response surfaces. For selecting the 'best' regression equation: there are two contradictory criteria for selecting a resultant equation: (1) to make the equation useful for predictive purposes we should want our model to include as many

independent variables as possible so that reliable fitted values can be determined; and (2) because of the costs involved in obtaining information on a large number of independent variables and subsequently monitoring them, we should like the equation to include as few independent variables as possible (Draper and Smith, 1981). The independent variable is a function of one or more X variables. The selection of the best regression equation is a compromise between these two criteria.

The *best subsets regression using R^2, R^2 (adjusted) or Mallows Cp statistic* method appears to be the most useful with the type of data which will be acquired from this experiment, since a model driven response surface is desired. Biomedical Computer programs[4] (Dixon, 1981), specifically BMDP program 9R, allows for the selection of up to ten best subsets of predictor variables in regression, whereby the user specifies the number of independent variables in the full model and the selection criterion. The program then produces the 'best κ' subsets out of all possible regressions (2^{κ}) where the latter is dependent upon the number of independent variables [x + f(x)]. It will chose the 'best' subset based on the method the user defines, i.e. Mallows Cp statistic. Some drawbacks to this procedure include: (1) a tendency to provide equations with too many predictors; (2) if the κ is chosen to be too small, the most sensible choice of fitted equation may not appear in the overall 'best κ' subset, though it will appear elsewhere in the printout; (3) no printed information is readily available concerning how the various subsets were obtained. These problems can be overcome by first having a number,

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[4] Dixon, W. J. (ed.) 1981. *BMDP Statistical software 1981.* University of California Press, Los Angeles.

e.g. 10, of best equations based on low Cp values and secondly, creating a plot of Mallows Cp statistic versus the p value (K variables plus the intercept) for each equation and comparing each point to a Cp equal to p line. Any points above this 1:1 line are considered to be biased and any points below have random error involved. A 'point' represents the number of independent variables plus the intercept. The choice of equations now becomes easier because it is possible to visually chose an equation which is not biased and has the fewest predictor variables and the smallest random error by choosing an equation which is closest to the 1:1 line and the origin. If the user then runs BMDP program 9R again, this time using method 'none' and only the final subset regression equation, detailed statistics for the equation are obtained. This may be necessary only when the user chooses an equation which differs from the subset with the lowest Cp, which is likely. It is important to remember that multi-variable linear regression models are often satisfactory for the prediction of the response variate, but are gross approximations to the true models, which are likely to be non-linear (Ratkowsky, 1990).

HYPOTHESES

Delouche and Baskin (1973) pointed out that accelerated aging techniques can be employed to produce a series of sublots that cover the entire range of deterioration derived from one seed lot, and naturally aged seed was unavailable. The research discussed in this thesis is based on the following hypotheses:

1. Artificially aged maize seeds will respond as naturally aged maize seeds.
2. An effective indicator of maize seed quality derived from the electroconductivity of individual seed leachates can be found.
3. Determination of the index is not injurious to the seed sample.

MATERIALS AND METHODS

Electroconductivity tests

Soak time

Zea mays L. cv. B73*LH51 seeds, lots 88-1d (dryland) and 88-2i (irrigated), were donated by the National Seed Storage Laboratory (NSSL) located on the campus of Colorado State University, Fort Collins, Colorado. The decision concerning the appropriate soak time for maize seed was based on several runs and scans of leachate conductivity values from an ASAC-1000B for runs up to 24 hours, where runs times scans resulted in 319 observations. Data were combined and a plot of Internal Slope (IS) vs. time was created in SlideWrite[5] where $IS = at^{-b}$, a power function, was fitted, Figure 1. An arbitrary line was drawn at 5 hours when the leaching process was considered to have leveled off and stabilized. Based on these plots the soak time for all electroconductivity tests was reduced from 24 hours, the soak time recommended by the ASAC Manual, to 6 hours. Before deciding on 6 hours of soaking, some experiments employing an 11 hour soak were done but radicle emergence occurred in one of the seed lots.

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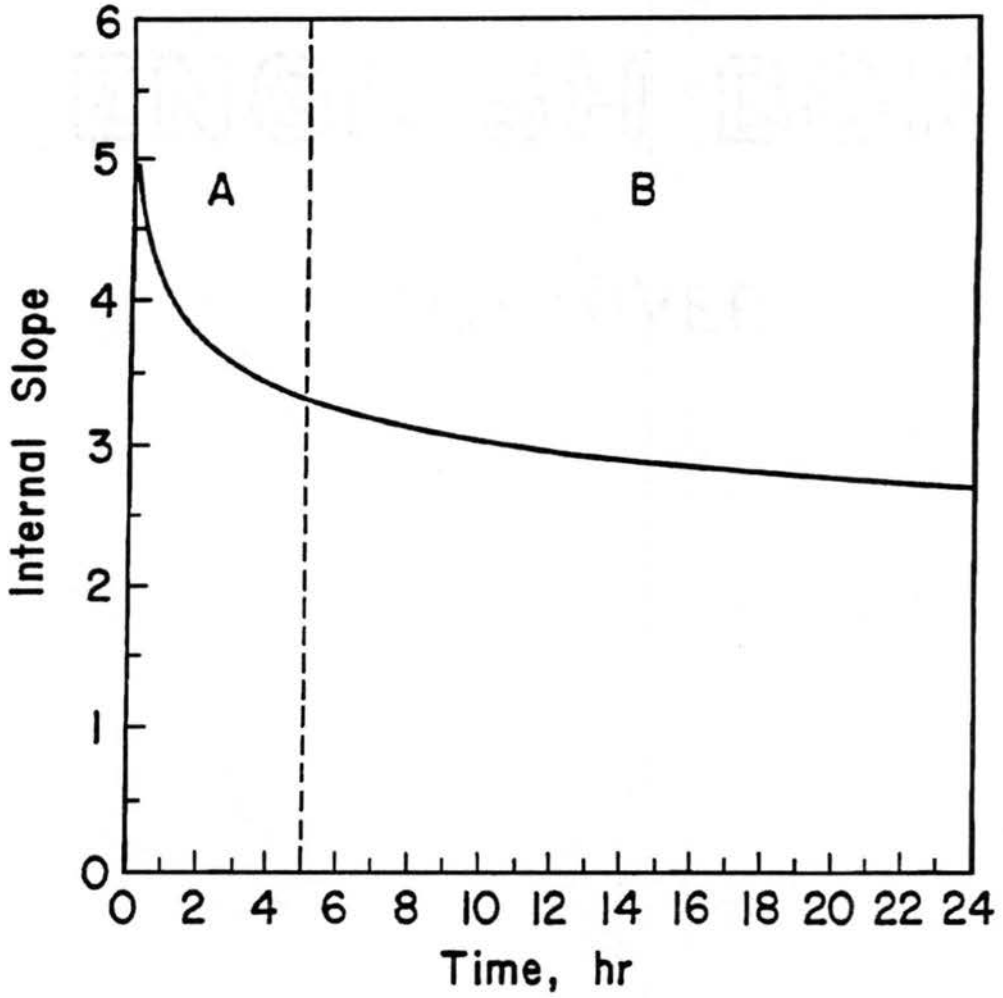
[5] Advanced Graphics Software, Inc. 1990. SlideWrite plus ver. 4.0 user's manual. Eighth ed. Program by Bessie Chin and Larry Daniele. Sunnyvale, CA, 94086.

Figure 1. Internal slope index in response to soak time for *Zea mays*. A represents the region of rapid leakage and instability. B represents the region of stability where the curve levels off.


Internal Slope
6
5
4
3
2
1
0
A
B
0 2 4 6 8 10 12 14 16 18 20 22 24
Time, hr

Aging Methods

After careful consideration of several aging methods (Bruggink, 1989; Delouche and Baskin, 1973; Matthews, 1980; Tao, 1979; Vertucci and Roos, 1991) two methods were chosen. Method A was designed after Bruggink's alternative controlled deterioration test and method B employs the Vertucci and Roos method. These two seed aging methods were used to obtain varying levels of seed viability.

Method A utilizes high moisture and high temperature for a short period of time to cause the seeds to deteriorate. Initial moisture content (MC) was determined by weighing 5 seeds of each lot, drying them at 105°C for 24 hours, reweighing them and calculating MC on a dry weight basis, e.g. MC = [(initial weight - dry weight)/dry weight] x 100. Samples from 2 seed lots, B73*LH51 88-1d (dryland) and 88-2i (irrigated) maize, were equilibrated to approximately 16% moisture (dry weight basis) over a saturated solution of NaCl, equivalent to 75% RH at 20°±1°C, in a large desiccator (Bruggink, 1989). Two desiccators were prepared each with 500 ml of saturated NaCl solution on the bottom and a shelf 5 cm above the surface of the solution. Several layers of cheesecloth were placed on the shelf of each desiccator to prevent the seeds from falling to the bottom of the desiccators, to allow for free movement of water vapor around the seeds and to provide an adequate method of removing seeds from the desiccators. Both desiccators were kept at 20°C on the bottom shelf of the germinator. Each seed lot was contained in a separate desiccator. The samples within these desiccators were stirred every 4 to 6 days. Moisture content, where 10 seeds were weighed oven dried for 24 hours at 105°C and reweighed, was calculated after 6 and 13 days and

determined to be about 12% and 14 to 15% for 88-1d and 88-2i lots, respectively. Once the seeds reached an equilibrium, after 16 to 20 days, of 16% moisture they were removed from the desiccators and samples of 110 seeds/packet were sealed in individual aluminum foil packets. A moisture content of 16% (dry weight basis) is approximately double their storage moisture content. These were stored at room temperature, 22°C, until they were aged for 120 hours at 45°C. The number of seeds sealed into each packet allowed for 100 test seeds and 10 seeds for moisture determination. The seeds were aged for 120 hours at 45°C in a convection oven in an attempt to obtain germination of about 70%.

The approximate number of seeds used for each lot was 1100 for a total of 5 replications for each seed lot. One replication consists of 100 seeds soaked to obtain μAmp data, 100 seeds for a paired germination test and 10 seeds for MC determination. Moisture content determination seeds were soaked at the same time as the electroconductivity tested seeds so that the weight of the MC seeds could be monitored to know when the 100 soaked seeds attained their original moisture content. After soaking, prior to germination, the seeds were air dried at room temperature for five to seven days.

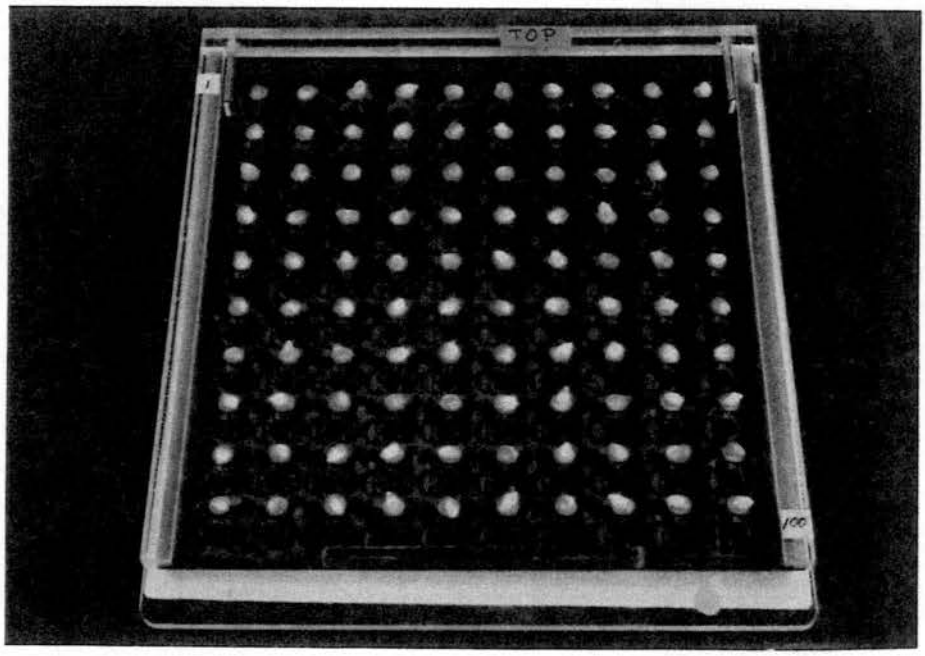
Method B utilized low moisture and high temperature for a long period of time (Vertucci and Roos, 1990). Two samples of approximately 2500 seeds, each in it's own desiccator, were held over H_2SO_4 to obtain a very low moisture of approximately 1% (dry weight basis) and aged at 35°C for 238 or 484 days, for desiccator 1 and 2, respectively. After aging was complete the seeds were placed in another desiccator over a saturated NaCl solution at room temperature, 75% RH, for 10 days until the moisture content was up to 7% moisture (dwb) to avoid the

problem of severe imbibitional injury (Nutile, 1964; Ellis *et al.*, 1990). The samples were kept in aluminum foil packets until tested. Unlike Method A, the seeds from desiccators 1 and 2 were all put into packets 1 and 2, respectively, which were closed with a clip.

Naturally aged seed was not used because varying degrees of viability of each variety was required. Naturally aged seed that had very low viability levels was unavailable.

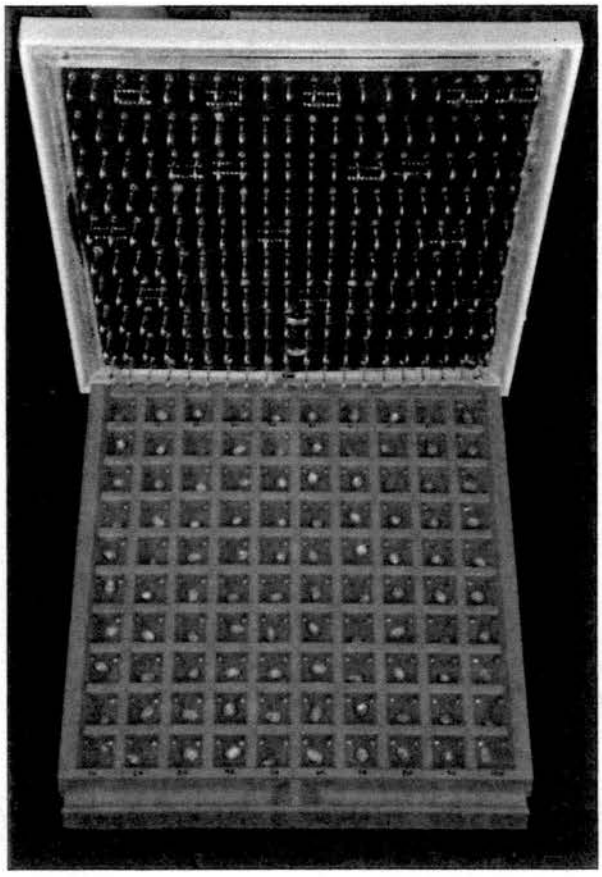
Prior to electroconductivity testing, the PC and ASAC-1000B were turned on to warm up for about 30 to 60 minutes. During this time the 10 moisture determination seeds were weighed and their weights recorded. One hundred test seeds were placed in a seed counting tray (Figure 2), a device which fits directly over the soaking tray and allows all 100 seeds to be dropped into the soak water simultaneously. The seeds were handled with forceps to prevent manual transfer of electrolytes. Once this was done the soaking tray was rinsed, filled with deionized water and the multi-electrode head placed onto it to test the tray and the ASAC-1000B for electrolytic and electronic cleanliness, respectively. Before testing could be done, the computer and the ASAC programs had to be activated. The ASAC-1000B was initialized for the time, date and voltage (1 volt for maize) by pressing the F3 key and entering the correct information. After this, pressing F6 prompted questions for terminal vs. CPU connection to the PC, terminal was chosen, and BAUD rate, 9600 was chosen. The PC was prepared by starting the ASACSTAT program. This was easily done by typing 'asa' from the floppy disk drive prompt where the data were to be stored. A message read 'make sure ASA1000B is set to TERMINAL mode with BAUD = 9600'. Pressing 'return'

Figure 2. Seed counter tray with 100 maize seeds in place. This device allows all of the seeds to be dropped into the soaking tray simultaneously.


TOP

brought up the 'Conductivity Scanning Program' menu. Testing of tray and water was done by choosing #7, 'test the ASAC-1000B', from the ASACSTAT menu on the PC. If an all zeros readout was obtained it was concluded that the soaking tray and multi-electrode head were both clean. If μAmp readings above zero were evident then the tray was rinsed and retested until all zeros were obtained. Any cell repeatedly displaying a high μAmp reading indicates that the multi-electrode head needs to be electronically cleaned and when several cells have high μAmp readings the tray or deionized water was suspect. The clean tray was then emptied and each of the 100 cells were filled with 2 mls of deionized water using a repipettor which was calibrated to 2 ml with 5 pumps of water into a 10 ml volumetric flask. The tray was tested one final time and the seed counter tray was positioned, with seeds in place, over the soaking tray so that each seed was located over a 2 ml cell of deionized water. Choosing number 3, from the ASACSTAT menu, 'Automatic scanning with individual input', provided several prompts for information: 1) enter a description for this data's scan in 70 characters or less, 2) enter a filename for this data's scan without adding an extension because this was automatically done, 3) enter up to 4 separate sampling rates (enter the sampling interval in minutes then enter the period duration in minutes and 4) place the scanning head (multi-electrode head) on the sample and press return to begin scanning. Figure 3 illustrates the soaking tray with one maize seed per 2 ml deionized water per cell and the multi-electrode head. The soaking tray and multi-electrode head were put into a covered constant temperature waterbath chamber, Blue M Electric Company Magni Whirl® (Blue Island, Illinois), 4 cm above

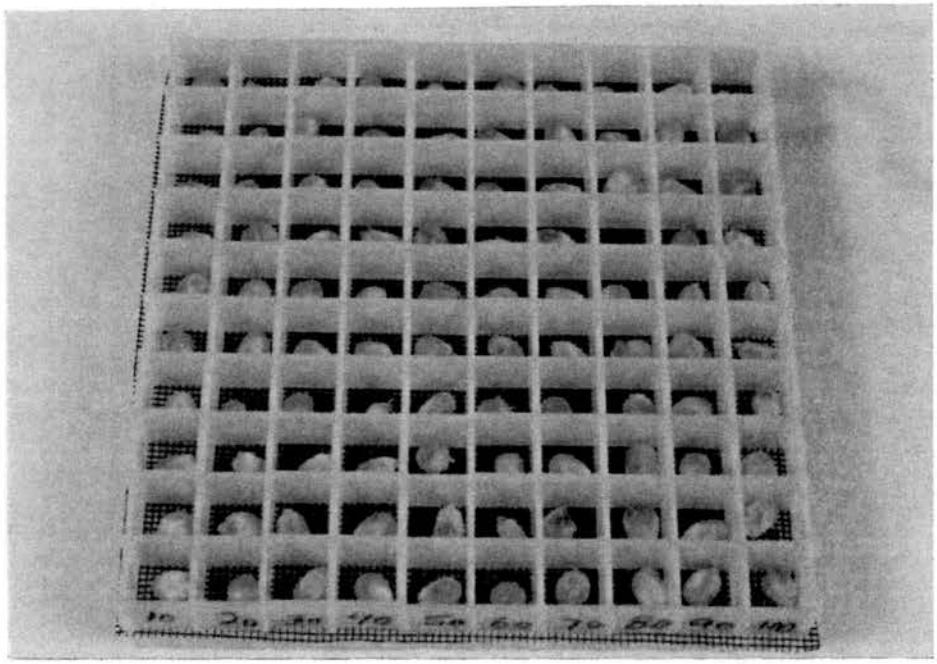
Figure 3. The seed soaking tray with multi-electrode head. Each cell of the soaking tray contains 2 ml of deionized water and one maize seed. One pair of electrodes fits into each of the 100 cells of the soaking tray.



the surface of the water and kept at 25°C to eliminate the effect of temperature change on leakage rate as well as to prevent evaporation (Bruggink *et al.*, 1991).

Each aging method replication, 100 seeds per tray with 1 seed per 2 ml of deionized water per cell, was soaked for 6 hours. There were 29 scans made during the 6 hour soak period, constituting one run. One scan consisted of 100 conductivity readings at an instant in time. Ten additional, pre-weighed seeds for moisture determination were soaked at the same time to determine when the test seeds regained their original moisture level. Electroconductivity of seed leachate was measured in μAmps/seed by an ASAC-1000B multi-electrode seed analyzer at a setting of 1 volt. A modified version of ASACSTAT (NSSL) was used to automatically collect μAmp readings at pre-designated times and store them to a file on the PC for later manipulation. Scan times were set at every 5 minutes for the first 30 minutes followed by every 15 minutes for the remaining 330 minutes. The seeds were then removed with forceps, placed into specially designed drying trays, Figure 4, and air dried on the lab bench for about 7 days or until initial moisture content (dry weight basis) was regained. This was determined by weighing the MC determination seeds every 3 to 4 days. Moisture determination seeds were oven dried for 24 hours at 105°C. When the seeds regained original MC, germination was done by the rolled paper towel method (AOSA, 1988). One hundred unsoaked (control) seeds were germinated at the same time. After 72 hours, the seeds were removed long enough to measure radicle lengths (mm) and returned to complete the required seven days of germination.

Figure 4. A Drying tray with 100 maize seeds which was specially designed to allow free air movement around each seed and to track each individual seed.



Each scan time was saved to an individual file, all of which were concatenated into one file containing 100 electroconductivity values for every scan time. This was done in the DOS[6] program by copying all of a particular set of files to a different filename. After this was done, there were some minor alterations which had to be made to the concatenated file for it to be accepted by the Richards program. The Richards program allows for a five character title for each scan so the DOS input file was manipulated in WordPerfect 5.1[7] to remove quotation marks and to create headings for each scan time. It is important to remember to save the file in DOS or ASCII format after manipulation. Each file was then transferred to an IBM RS6000 model 530 called OPS (AT&T Starlan running Microsoft Lan Manager) onto the user's account on the CSUNet using the file transfer protocol (ftp). In order for the ftp program to automatically transfer the DOS file into UNIX format, ftp had to be initiated from the DOS side of the program i.e. to initiate transfer from the floppy disk drive where the data were stored. The Richards program is compiled into a file called 'a.out' on the user's account in OPS. Typing 'a.out input filename > output filename' runs the file through the Richards program to obtain the values of Internal Slope (IS), mean (trimmed) and median μAmps over the time course of leakage. Figure 5 is an illustration of a flow chart which visually describes the above mentioned procedures.

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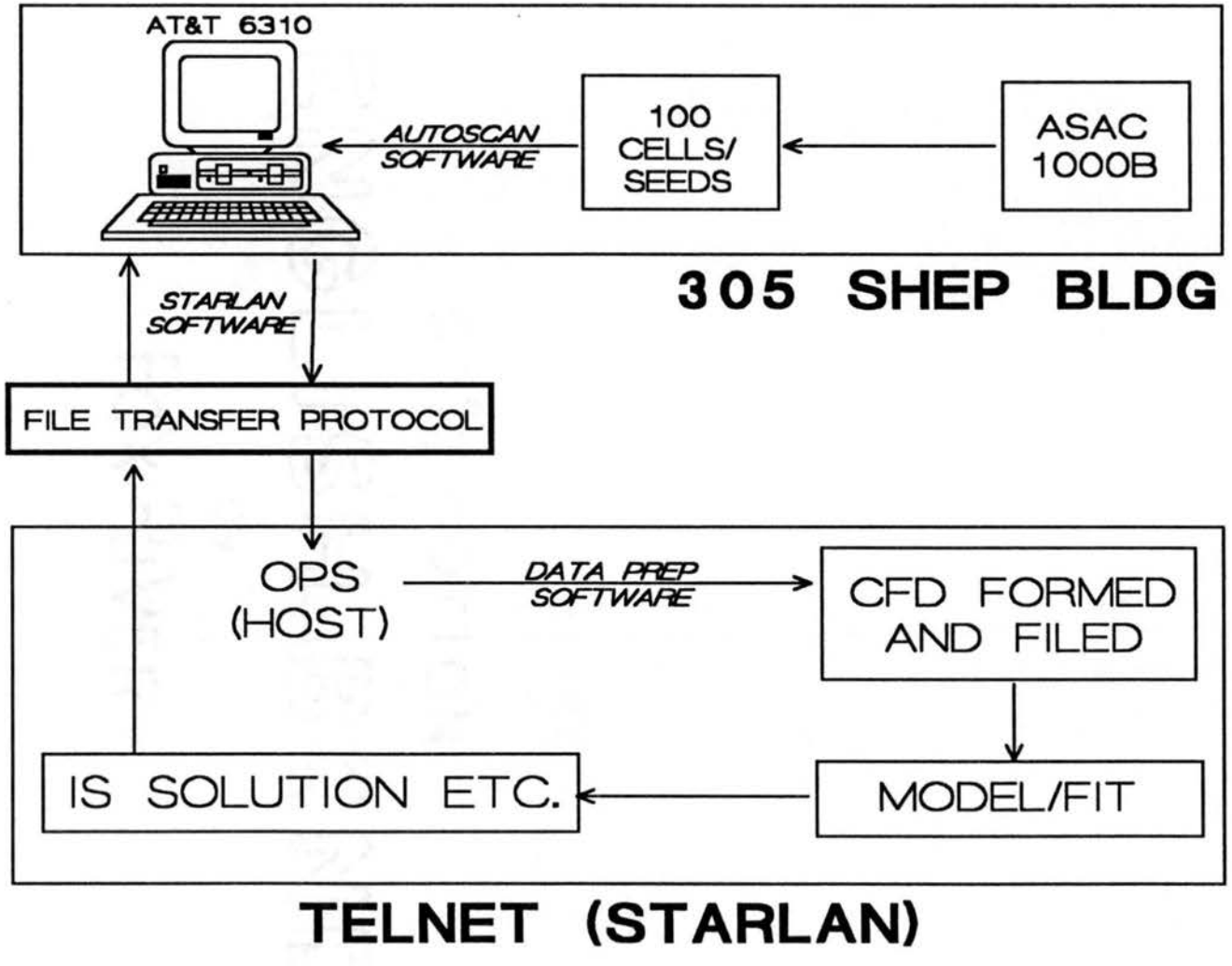
[6] Firenze Research Lab. Microsoft MS-DOS Operating System version 5.0. 1991. User's Guide and Reference.

[7] WordPerfect Corporation. 1990. WordPerfect version 5.1. 1555 N. Technology Way. Orem, Utah 84057 U.S.A.

Figure 5. Flow chart of data acquisition, file transfer and Richards function fitting at Colorado State University.


AT&T 6310
AUTOSCAN SOFTWARE
100 CELLS/ SEEDS
ASAC 1000B
305 SHEP BLDG
STARLAN SOFTWARE
FILE TRANSFER PROTOCOL
OPS (HOST)
DATA PREP SOFTWARE
CFD FORMED AND FILED
IS SOLUTION ETC.
MODEL/FIT
TELNET (STARLAN)

Several functions were fitted to the conductivity data: first the Richards function (Nath *et al.*, 1990) to obtain the mean and median μAmps and internal slope (IS), then the two parameter rectangular hyperbolic function (Johnson, 1987) to obtain the initial leach rate (ILR) and finally another version of the Richards function (Garrett *et al.*, 1989; Nath and Moore, 1992; Nath *et al.*, 1993) to obtain the average absolute leach rate (AALR). Where F is cumulative frequency and μAmps is current carrying capacity i.e. conductivity of single seed leachate solutions, *t* is soak time and *A, k* and *N* are Richards function parameters where

$(dF/d\mu Amps)_{max}$ at 5 hours.....IS

$d\mu Amps/dt$ at 0 hours.....ILR

$Ak/2(N+2)$.....AALR

represent IS, ILR and AALR (Causton and Venus, 1981), respectively. To determine IS values, cumulative frequency is regressed on cumulative μAmps [total frequency is 100 (seeds)] determined at the end of a 5 hr soak period. Primary data for both initial and average leach rates were cumulative μAmps versus soak time. In summary, the internal slope index is derived 'at an instant', whereas the candidate indices, ILR and AALR, are derived based on time dependent cumulative μAmp data.

The power function, in the form $IS = at^{-b}$ where *t* is soak time and *a* and *b* are constants, was fitted to IS vs. time data using SlideWrite Plus. Johnson's, 1987, version of the rectangular hyperbola,

$$\mu Amps = \frac{At}{Am+t} \qquad (9)$$

where t is soak time, A is the upper asymptote and m is a constant, was fitted to mean μAmps versus t data to obtain ILR, calculated as $1/m$, using SigmaPlot 5.0[8]. Another file of the mean μAmp values vs. t was created, in WordPerfect 5.1, to run through the second Richards function (Garrett *et al.*, 1989; Nath *et al.*, 1993) program (also on CSUNet). The average absolute leach rate (AALR) is calculated by this program. Analysis of each of the possible indices of maize seed quality was done utilizing linear regression, Table 1.

Viability and Vigor Loss

A single cross hybrid produced under irrigated conditions, Pioneer 3541 (also donated by National Seed Storage Laboratory), lot 88-9 initial germination of 96%, was used for a simulation of multiple electroconductivity tests. Initially 5500 seeds were soaked in deionized water (Table 2) and divided into multiple treatments, five cycles (C) and five cycle durations (CD). There were two replications, the first contained two seed moisture (SM) levels as a variable. 'High' moisture seeds, from replication one, resulted from placing the sample in a desiccator over a saturated NaCl solution for one day prior to germination and vigor testing. Since the results from the first replication indicated that seed moisture had no effect on viability, and it's effect on vigor was uncertain, it was decided not to include it as a variable in the second replication.

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[8] Jandel Scientific. 1992. SigmaPlot scientific graph system ver. 5.0 user's manual Program by J. Norby, S. Rubenstein, T. Tuerke, C. S. Farmer, R. Forood and J. Bennington. San Rafael, CA, 94901.

Table 1. Linear regressions performed on each of the candidate indices for maize seed quality.

% Germination on:	Relative Vigor on:
IS at t = 5 hours	IS at t = 5 hours
Mean μAmps at t = 5 hours	Mean μAmps at t = 5 hours
Median μAmps at t = 5 hours	Median μAmps at t = 5 hours
ILR at t = 0 hours	ILR at t = 0 hours
AALR	AALR

Table 2. Seeds per treatment per replication. Total seeds per replication were 3750 per moisture level.

Cycle	Cycle Duration, Hours Soaked					Vigor Test Control (Relative Vigor)
	2	4	6	7	8	
1	500 40[9] 10[10]	500 40 10	500 40 10	500 40 10	500 40 10	200
2	400 30 10	400 30 10	400 30 10	400 30 10	400 30 10	200
3	300 20 10	300 20 10	300 20 10	300 20 10	300 20 10	200
4	200 10 10	200 10 10	200 10 10	200 10 10	200 10 10	200
5	100 0 10	100 0 10	100 0 10	100 0 10	100 0 10	200

[9] Moisture determination seeds to be resoaked in successive cycles.

[10] Seeds consumed for moisture determination, 10 per cycle per cycle duration.

Subsamples were held for 5 to 7 days between cycles, constituting a dryback. Evaluation of viability (% germination) and vigor (radicle length) was done at the end of each cycle for each C*CD treatment including both SM levels for replication one and consuming 100 low moisture and 100 high moisture soaked, i.e. treated, seeds plus 200 unsoaked (control) seeds (separated into samples of 100). Specifically, evaluation of germination involved placing seeds in rolled paper towels at 25°C for 7 days (AOSA, 1988). There was no correction for percent germination caused by the presence of abnormals. Relative vigor based on radicle length was measured in millimeters after 72 hours and calculated as length of treated seeds/length of control seeds. Seed moisture content for each CD was determined at the end of each cycle by first weighing 10 C*CD seeds for each of the two moisture levels (replication one) and drying them at 90°C for 5 days, reweighing and calculating % moisture on a dry weight basis. This protocol was repeated until all 5 cycles were completed. Additionally, the second replication consisted of weighing the MC of 10 seeds after the imbibition period for each C*CD treatment, called imbibed MC and determined on fresh weight basis (fwb).

Biomedical Computer Programs (Dixon, 1981), BMDP, provides an algorithm, Program 9R, which searches among all possible (2^{κ}) subsets and selects, using Mallow's Cp criterion, the 'best' 10 where κ above equals the number of independent variables including functions i.e. squares, cross products, logarithms, inverses and powers associated with the largest and/or complete model postulated. The Cp statistic is said to measure total squared error associated with a fitted subset with p terms where $p = \kappa + 1$. Cp can be defined as follows

$$Cp = \frac{RSS_p}{s^2 - (n - 2p)} \qquad (11)$$

where *RSS* is the residual sums of squares of a subset with p terms, s^2 is the residual mean square from the largest and/or complete equation and n is the number of observations i.e. data cases in the data set.

Five C's and five CD's, as well as two SM levels one averaging 10% and one adjusted to an average of 18% (dry weight basis) provided initially a 5*5*2 factorial experiment. The difference in SM was not significant for replication one, at $P<0.05$, resulting in a 5*5 matrix when the data were combined. Replication two consisted of a SM level of 10% resulting in a single 5*5 treatment matrix. Statistical analyses used were factorial ANOVA (Freed *et al.*, 1987) using MSTATC[11] and all subsets regression using BMDP program 9R (Dixon, 1981). The input data file contained a separation between replications which was initially included as a variable. However, replication was not included as a variable in the ten best subset models for either viability or relative vigor and so the data for both replications were combined, low SM (averaging 10%) data from the first replication and all of replication two (SM averaging 10%), and the 'best' models were chosen from a 2 factor 3rd order complete model with $\kappa = 9$, where κ is the number of independent variables (Draper and Smith, 1981). The independent variables for both initial 'full' models were CD, C, CD^2, C^2, CD^3, C^3, CD*C, CD^2*C and CD*C^2.

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[11] Freed, R. et al. 1987. User's guide to MSTAT. Crop and Soil Sciences and Agricultural Economics; Michigan State University.

Cp (defined in literature review) criterion (Dixon, 1981; Draper and Smith, 1981) including consideration of bias and random error (Daniel and Wood, 1980) were used in the selection of the final viability and relative vigor models. In addition, BMDP method 'none' was selected and using only the variables included in the final models gained in depth statistics, such as contribution to r^2, for each of the variables. After this the models were visually constructed using SigmaPlot 5.0, whereby 3-D scatter plots with drop lines presented the primary data followed by corresponding regression surfaces for both viability and relative vigor.

The final visual analysis of each C by CD treatment was accomplished by plotting the two responses, viability and relative vigor, each against the total number of soak hours, taking into consideration of both the number of cycles and the duration of each cycle. Linear regression was performed to better define the relationship.

RESULTS AND DISCUSSION

Electroconductivity Tests

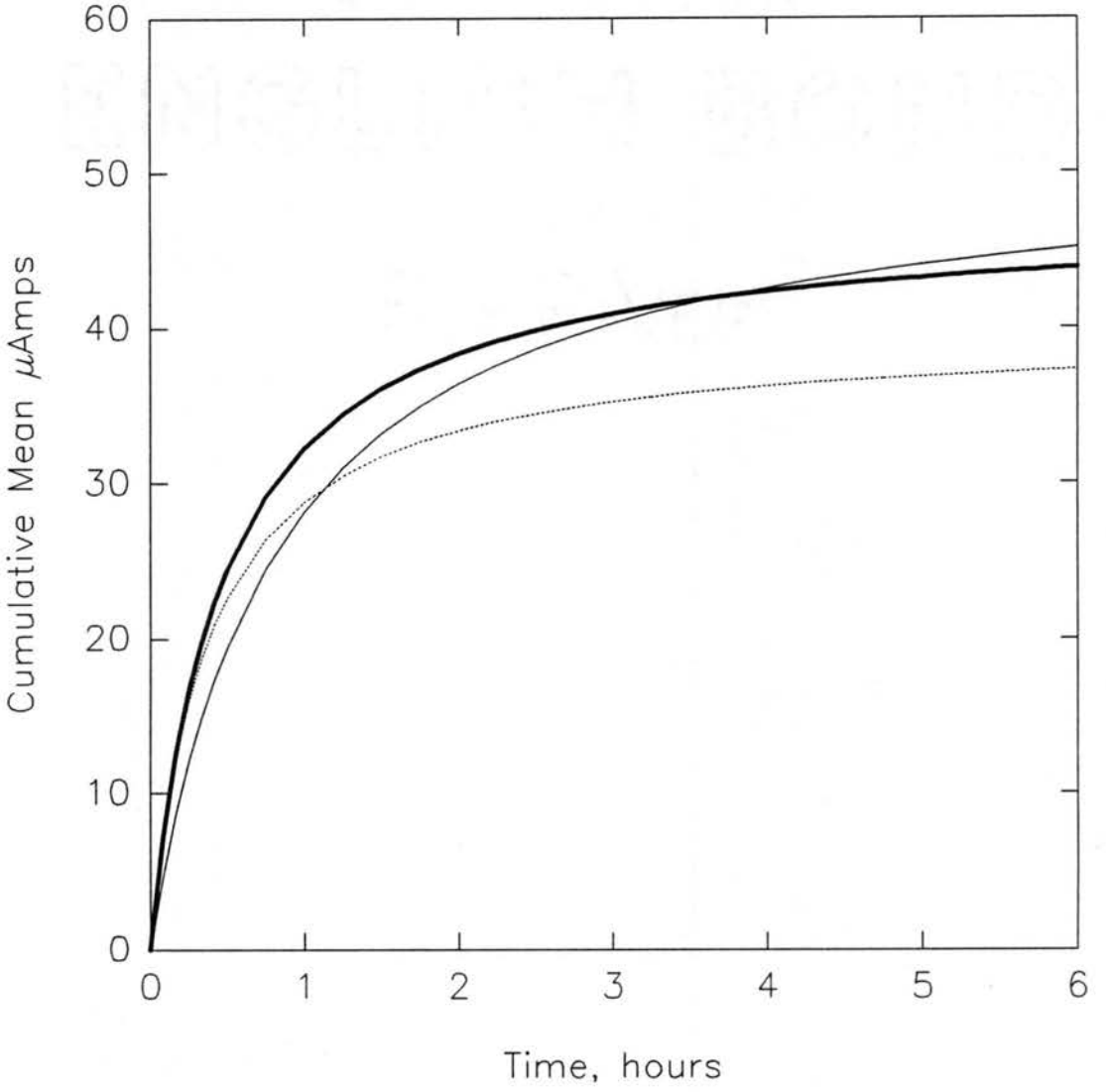
Soak Time

The rate of ionic exchange between a seed and the soak water varies throughout the soak period but eventually a point is reached whereby the release of exudates from the seeds slows dramatically or even stops. As the ion concentration stabilizes, so does the current value (μAmps), due to their close positive relationship. Initially the maize seeds were soaked for 24 hours since the ASAC manual recommends 18 to 24 hour soaks for hybrid maize seeds. It was soon discovered that radicle emergence occurred with such long soak periods. Soak time was reduced to 11 hours, but radicle emergence still occurred. Radicle emergence which occurs during the soak period is obviously destructive and so the soak period was reduced to six hours. There appears to be less destructive consequences to the seeds since there was no protrusion of the radicle occuring with this length of soaking. The less time seeds are soaked the less destructive the test is, which leads to the possibility of further reduction of the soak period for future testing.

Comparison of Aging Methods

Two aging methods were initially employed to obtain different levels of seed quality (viability and relative vigor) for comparisons. A replication refers to a sample of 100 seeds that were soaked, dried back and germinated, plus 100 control seeds which were not soaked. The experiment for Method A seed aging was repeated once. Method B seed aging was not repeated because the length of time required was too great. When the aging process for Method B was begun it was expected to last approximately two months with the seeds in conditions of high temperature with very low moisture content (Vertucci and Roos, 1990) at which time the seeds would lose viability and vigor. This was not the case, however. There were two desiccators of roughly 2500 seeds to be aged using Method B. Periodic checks were made to monitor the level of deterioration in each of the desiccators. One desiccator of seeds was removed from aging after 238 days and the other desiccator was removed after 484 days. There were ten replications each of 100 Method A aged seeds, for both the initial and repeated experiment and 17 replications each of 100 Method B aged seeds for each aging duration, 238 and 484 days, respectively. The lines in Figure 6 were obtained by entering the data for each replication and fitting the rectangular hyperbola (Johnson, 1987) to the combined data producing three lines; one for 10 combined replications of Method A, one for 17 combined replications of Method B aged 238 days and one for 17 combined replications of Method B aged 484 days. The repeated experiment for Method A was not included here because these data are not discussed further except to explain why they are not used. Figure 6 compares not only the two methods but also the difference in duration for method B. Method

Figure 6. The solid thin line represents 10 replications of 100 maize seeds which were aged by Method A, high temperature and high seed moisture. The solid thick line represents 17 replications of 100 maize seeds which were aged by Method B, high temperature and very low seed moisture, for 238 days. The dotted line represents another 17 replications which were aged by Method B for 484 days.


60
50
40
30
20
10
0
Cumulative Mean μAmps
0
1
2
3
4
5
6
Time, hours

B (238 and 484 days) have apparently the same initial rates of leakage, although Method B (484 days) had an overall lower current value which is not expected since a more severe aging regime should evoke greater electrolyte leakage (Ching and Schoolcraft, 1968; Gill and Delouche, 1973; Harrington, 1973; Bondie*et al.*, 1979; McDonald and Wilson, 1980; Ghosh *et al.*, 1981; Steere *et al.*, 1981; Keys, 1982; Saxena *et al.*, 1985; Powell, 1986; Furman, *et al.*, 1987; Pandey ,1988; Bruggink *et al.*, 1991; Nath *et al.*, 1991). Method A had an initially slower rate of leakage but the same amount of total leakage as Method B (238 days) after six hours of imbibition and appears as though it may continue to rise.

The first Method A aging experiment provided the desired outcome of several levels of viability, ranging from 0% to 89% germination. Radicle lengths ranged from 0 mm to 28 mm. Relative vigor ranged from 0 to 2.04. Relative vigor is the ratio calculated as the average radicle length, mm, of 100 soaked (treated) seeds divided by the average radicle length, mm, of 100 unsoaked (control) seeds; therefore it has no units. It provides for simple comparison between replications in the event that the same germinator was unavailable, not working, or there were temperature fluctuations during the germination period. The ranges of viability and vigor observed for Method A, first experiment, were probably caused by differences in seed moisture content prior to aging and/or position in the convection oven which was used for aging. Even though the method used was the modified controlled deterioration method (Bruggink, 1989) variations in seed moisture, from seed to seed, still occur. The second aging experiment for Method A, however, yielded a narrow range of viability, only 0% to 14% germination. The probable cause for the difference between the first and second experiments is temperature

fluctuations in the oven caused by power surges which occurred in the early morning hours, during the second experiment. Similar problems occurred with other equipment in the same laboratory during the time of the second experiment. As a result, the data from the repeat of the Method A were not considered further.

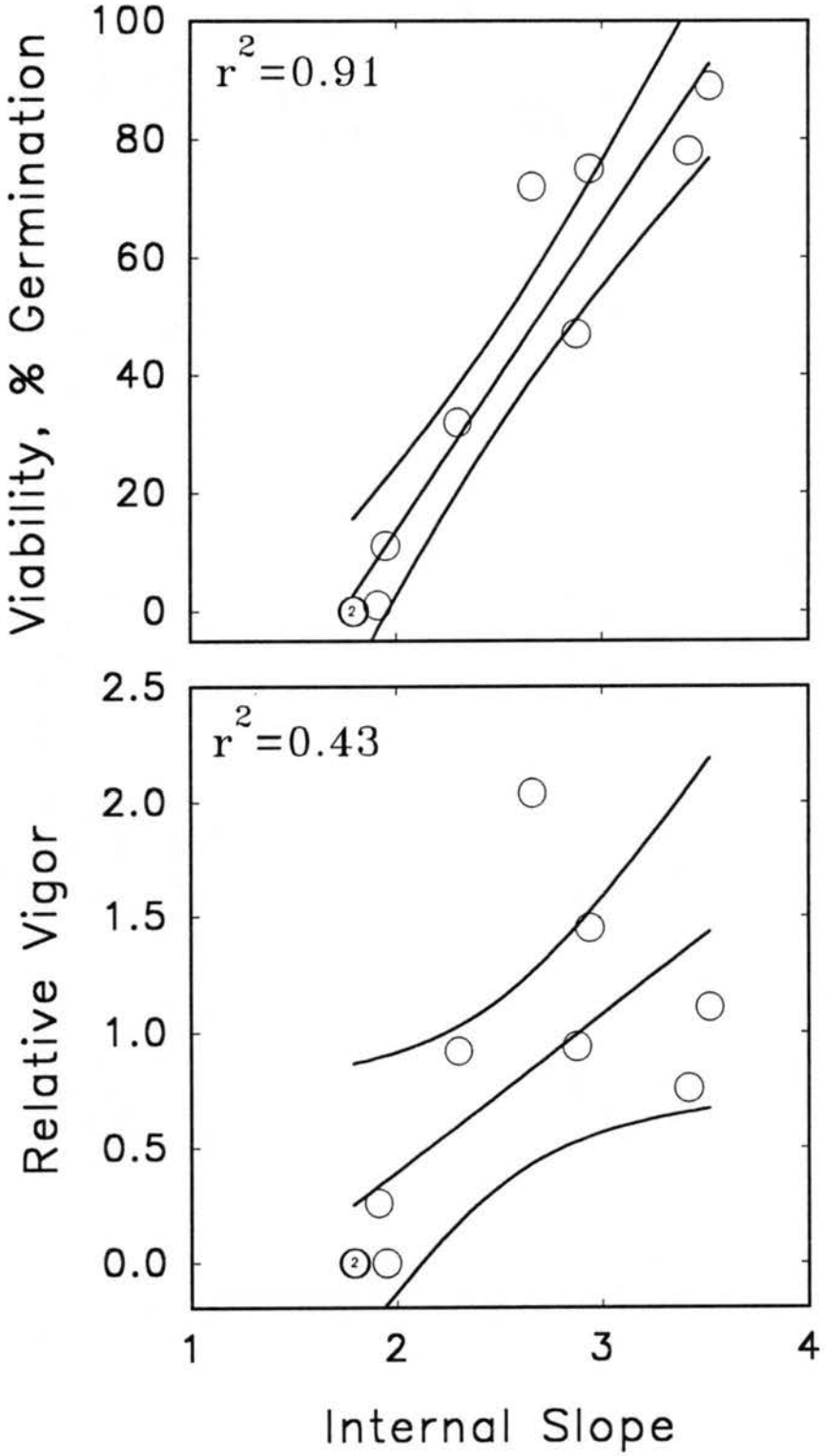
The Method B aging experiment consisted of two durations, 238 or 484 days. Even though the aging environments were the same, the seeds subjected to the longer duration produced a more desirable range of viability, from 61% to 92%, than the shorter duration, ranging from only 89% to 99%. Seed-to-seed variation in pre-storage histories can alter the results in artificially aging seeds. Also, it may be that the ranges observed were caused by differences in moisture contents of individual seeds due to their proximity to the sulfuric acid. Overall, Method B did not produce the range of viabilities that were expected and therefore only the results from the first method A aging experiment are presented and analyzed in this section. Upon germination of seeds from this method of aging there were many abnormal, short and stubby roots and shoots, just as Nutile (1964) observed.

Measures of Maize Seed Quality

It has been recognized that an inadequate spread of data points can easily distort the parameter estimates and/or give large standard errors of the estimates when fitting non-linear regression models to data (Causton *et al.*, 1978; Causton and Dale, 1990). It is assumed that there is an adequate spread of points in the present data for efficient curve fitting. Five indices were considered as possible predictors of maize seed quality (viability and relative vigor): IS (Moore *et al.*, 1988) after five hours of imbibition, the mean and median (Wilson, 1992) of 100 individual seed leachate conductivities after five hours of imbibition, the ILR index calculated at zero soak time from the rectangular hyperbola (Johnson, 1987) and the AALR (Garrett *et al.*, 1989; Nath *et al.*, 1993). Two linear regressions were performed for each of the candidate indices, one with viability as percent germination and the other with relative vigor based on radicle length as the dependent variable (Table 1).

The best predictor of viability, according to the regressions in Figure 7, was IS, with a positive $r^2 = 0.91$. The Richards function is fitted to the cumulative frequency distribution of μAmps formed by special purpose software, Figure 5. Internal slope is derived from the four Richards function parameters at an instant in time and the reciprocal internal slope, RIS, is directly related to within sample variability (Moore *et al.*, 1988). Internal slope accounted for only 43% of the variability when predicting relative vigor, Figure 7. Correlation coefficients for both viability and relative vigor were significant at $P < 0.05$. Part of the problem of using IS as an index of seed quality is that it changes over the soaking time course.

Figure 7. Linear regressions, with 95% confidence bands, of viability and relative vigor on internal slope index after 5 hours of imbibition; n = 10 scans each of 100 aged maize seeds.

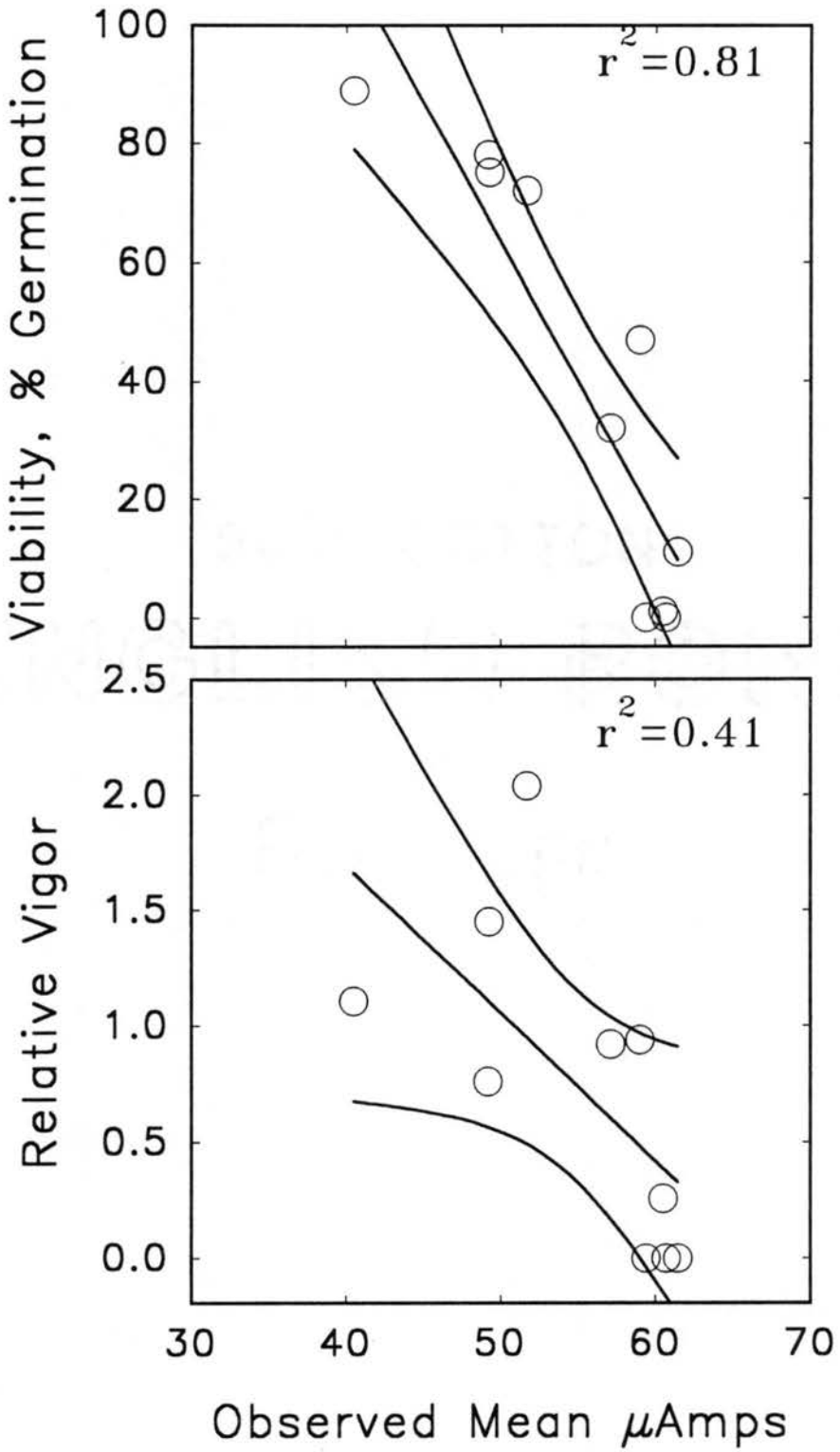

Viability, % Germination
100
80
60
40
20
0
r^2=0.91
Relative Vigor
2.5
2.0
1.5
1.0
0.5
0.0
r^2=0.43
1
2
3
4
Internal Slope

The slope changes rapidly at the beginning of the time course and then the rate of change slows down at later times. It would be beneficial to choose an IS value at a time where there is little change, but that point in time might be such that the seed germinates or loses too many solutes for normal germination. So it is necessary to choose a point in time when very little change occurs in the internal slope value with time. The initial phase of leakage is unstable (Duke *et al.*, 1983), therefore, it was necessary to choose a later, more stable time to access data for comparisons. This is possible because IS is sensitive to the duration of the test, i.e. soak period, Figure 1. Internal slope appears to be most sensitive to the first three hours of imbibition.

McDonald and Wilson (1980) demonstrated that μAmps per seed values increased from 69 to 148 with increased aging, which clearly indicates that the ASA-610 (the predecessor to the ASAC-1000B) monitors changes in soybean seed quality as a result of both mechanical damage and accelerated aging. As seeds age, quality level declines and the frequency distribution curve of seed quality changes from a well defined peak, at days zero and one, to a much wider curve, after days three and four, depicting a change in seed lot uniformity and quality (McDonald and Wilson, 1980). Thus, internal slope can accurately predict seed viability because it retains the idea of shape (Moore *et al.*, 1988).

Two of the candidate indices tested were measures of central tendency of the frequency distribution, the mean and median μAmp values of 100 individual maize seed leachates after five hours of imbibition. Figure 8 reveals that 81% of the variability in germination is accounted for using the mean (trimmed) μAmp value. Yet only 41% of the variability in relative vigor was accounted for. Both

Figure 8. Linear regressions, with 95% confidence bands, of viability and relative vigor on mean current value, μAmps, observed after 5 hours of imbibition; n = 10 scans each of 100 aged maize seeds.


Viability, % Germination
100
80
60
40
20
0
r^2=0.81
Relative Vigor
2.5
2.0
1.5
1.0
0.5
0.0
r^2=0.41
30
40
50
60
70
Observed Mean μAmps

correlation coefficients in Figure 8 were significant at $P < 0.05$. There was a negative relationship between both viability and relative vigor with the mean μAmp value after five hours of imbibition; the mean μAmp value increased as both viability and relative vigor decreased. This follows the principle of leakage studies since the concept of the electroconductivity test is based on the hypothesis that the amount of seed deterioration is expressed by a proportional loss of cell membrane integrity. For example, a non-viable seed leaks more electrolytes than a healthy, viable seed and a high electroconductivity value correlates with low field emergence and low vigor (Powell, 1986). The mean μAmp value acts like the bulk method (Wilson, 1992) which cannot account for seed to seed variability or population distribution within a seed lot (Mullet and Wilkinson, 1979). This led to the consideration of the median μAmp value to represent a measure of central tendency, which is not as affected by extreme values as is the mean, that relies on the distribution of 100 individual seeds.

The same negative relationship was noticed for the median μAmp value with a slightly better prediction capacity than the mean. The median μAmp value after five hours of imbibition accounted for 86% of the variability in germination as illustrated in Figure 9, and 50% of the variability in relative vigor accounted for using the same index. Both correlation coefficients were significant at $P<0.05$ for the relationships of viability and relative vigor with the median μAmp value.

The fourth seed quality index considered was ILR (Figure 10), which was derived by fitting Johnson's, 1987, version of the rectangular hyperbola, see equations **(9)** and **(10)**, to μAmp versus t data for each replication and calculating ILR as 1/m. The rectangular hyperbola has biological rationale, it is frequently used

Figure 9. Linear regressions, with 95% confidence bands, of viability and relative vigor on the median current value, μAmps, observed after 5 hours of imbibition; n = 10 scans each of 100 aged maize seeds.

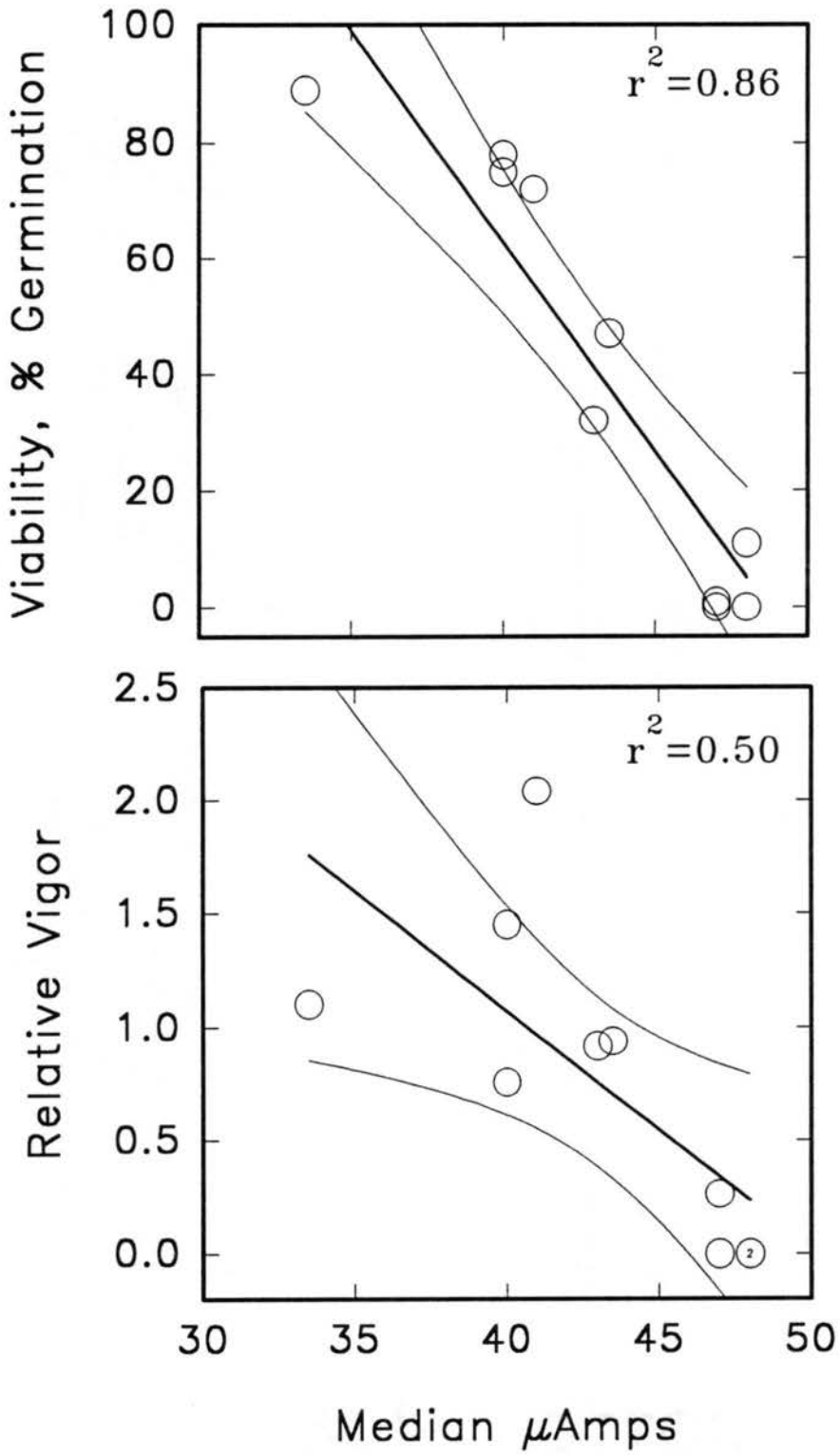
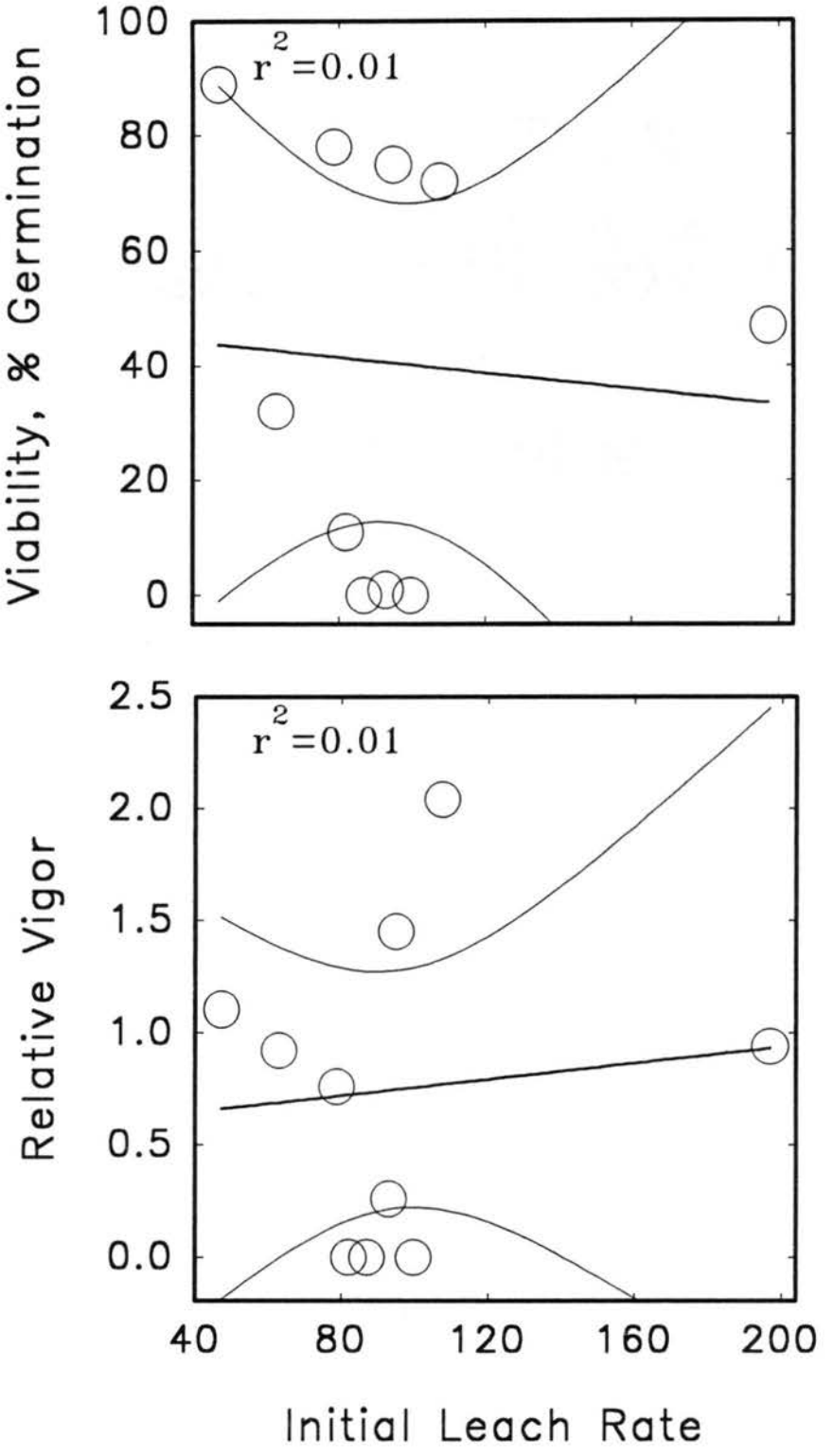

Viability, % Germination
100
80
60
40
20
0
r 2=0.86
Relative Vigor
2.5
2.0
1.5
1.0
0.5
0.0
r 2=0.50
30
35
40
45
50
Median μAmps

Figure 10. Linear regressions, with 95% confidence bands, of viability and relative vigor on the initial leach rate (ILR); n = 10 runs each of 100 aged maize seeds.


Viability, % Germination
100
80
60
40
20
0
r^2=0.01
Relative Vigor
2.5
2.0
1.5
1.0
0.5
0.0
r^2=0.01
40
80
120
160
200
Initial Leach Rate

in enzyme kinetics (Thornley and Johnson, 1990) and as such fits the leakage data very well. Figure 10 shows that there was no correlation between ILR and viability or relative vigor. ILR does not predict either viability or relative vigor. The ILR is the initial slope, a theoretical maximum rate at zero soak time, or the rate of leakage which occurs with the first contact with water. This is a highly variable state of imbibition, ranging dramatically from seed to seed depending upon moisture content of each seed prior to soaking. Duke *et al.* (1983) suggested that the leakage of electrolytes as a function of seed hydration is a passive process during the early phase of imbibition before the membranes have reorganized and reattained selective permeability. Keys (1982) interpreted the initial phase of rapid electrolyte leakage, which is non-linear, as physical and not physiological, therefore, not indicative of seed quality.

Keys (1982) also defined the linear portion after the first hour of imbibition to be a steady state controlled physiological phase indicative of seed quality. The final seed quality index examined was the average absolute leach rate, AALR, which can be defined as the integral mean of all leakage rates over the entire soak period and which is derived from the Richards growth function program (Garrett *et al.*, 1989), as opposed to the Richards seed function program (Nath *et al.*, 1990). The average absolute growth rate was adopted from the concept of change in a growth attribute per change in time as the change in leakage per change in time which is calculated as

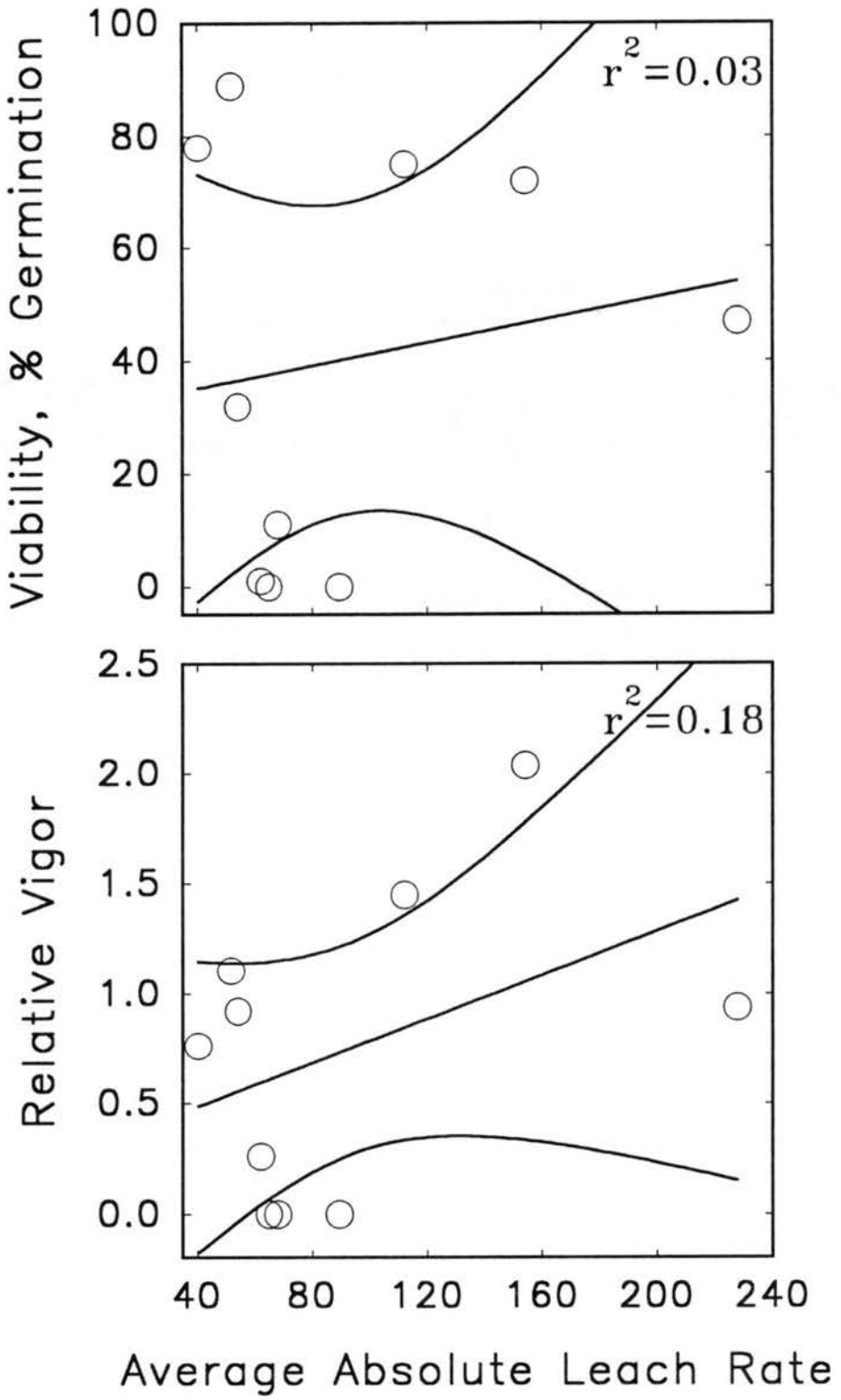
$$\frac{Ak}{(2(N+2))} \qquad (12)$$

from the Richards function equation **(13)** (Nath *et al.*, 1993) where *A* estimates the asymptotic maximum, *k* can be interpreted as a measure of the rate at which *A* is approached, *N* determines the location of the inflection point and *t* is time.

$$W=A(1\pm\exp(b-kt))^{-\frac{1}{N}} \qquad \textbf{(13)}$$

Figure 11 represents the linear regressions of AALR on viability and relative vigor, respectively. There was no correlation between either viability and AALR or relative vigor and AALR and neither correlation coefficient was significant at $P < 0.05$. The AALR does not represent a change in an actual growth attribute and this may be why AALR does not predict viability or relative vigor. A study by Kuo, 1986, suggests that once solute leakage has slowed after the early leachate was removed an effective prediction of seed quality could be made. This approach may improve the use of AALR as an index of seed quality.

Figure 11. Linear regressions, with 95% confidence bands, of viability and relative vigor on the average absolute leach rate (AALR); n = 10 replications each of 100 aged maize seeds.


Viability, % Germination
100
80
60
40
20
0
r^2=0.03
Relative Vigor
2.5
2.0
1.5
1.0
0.5
0.0
r^2=0.18
40
80
120
160
200
240
Average Absolute Leach Rate

Viability and Vigor Loss

To determine how destructive the electroconductivity test truly is, an experiment was designed to simulate multiple EC tests; however storage time was not accounted for in this experiment and should be considered further. There were two experiments performed utilizing the same procedures. These experiments involved soaking samples of 100 seeds for 2, 4, 6, 7 and 8 hours up to five times, i.e. there were 5 cycles of 5 durations for a total of 2500 seeds for each repeat of the experiment, 3750 including the unsoaked control seeds and moisture determination seeds (Table 2). A 5*5 treatment matrix was developed from each of the two sets of data. Multiple regression analysis using BMDP statistical software was performed on each of the data sets, individually and combined. The combined analysis, however, included replication as an input variable to distinguish between the two sets of data to determine whether there was a difference over time because visual observation of the primary data for the two individual experiments suggested that they could be effectively combined. The simplest method for testing this hypothesis was to combine the two data sets into one while maintaining their origin in time, i.e. distinction between replications one and two. If there had been a difference it would have manifested itself by presenting replication as a part of each of the ten best subset models chosen by the software. Since replication was not included in any of the ten best subsets, BMDP multiple regression analysis confirmed that the two sets of data were not significantly different and could, therefore, be combined to acquire the single best regression models for viability and relative vigor in response to the number of cycles and cycle

duration. The primary data for the two replications were combined and are presented in Figure 12 for viability and Figure 13 for relative vigor. These combined data sets, now n = 50 data cases instead of n = 25, were used to generate the 'best' models for viability or relative vigor.

Electroconductivity values were taken at the end of each cycle duration for each cycle, Table 3. These data reveal that about 45% of the readily leachable electrolytes leach from maize seeds in the first soak cycle, upon the first contact with free water. The membranes allow leakage each time the seeds are dried, not acting like retentive barriers until rehydration, i.e. the membranes lose their integrity each time they are dried but as hydration occurs the membranes become reestablished preventing further leakage (Simon and Raja Harun, 1977). It appears that the membranes of the seeds, which lose most of their electrolytes during the first hydration period, require less time to reestablish selective permeability after the first hydration period, Table 3. Weges and Karssen (1991) suggested that intact seeds do not leak during a second hydration period because nearly all of the potassium leaked out during the first hydration period and that this potassium originates near the testa preventing leakage from the embryo. Weges and Karssen (1991) observed that intact seeds as well as removed testae leached approximately the same amount of potassium ions with a single imbibition treatment; furthermore, testae which had undergone a cycle of imbibition and drying did not leach potassium ions upon re-imbibition, the same response was observed from whole seeds with the same treatment.

An interesting phenomenon occurs after the seeds are soaked for one cycle. Regardless of the length of soak, they lose much of their color and become a

Figure 12. Primary data from which the response surface for viability as a function of number of cycles and cycle duration was generated. Two replications were combined, providing n = 50 observations.

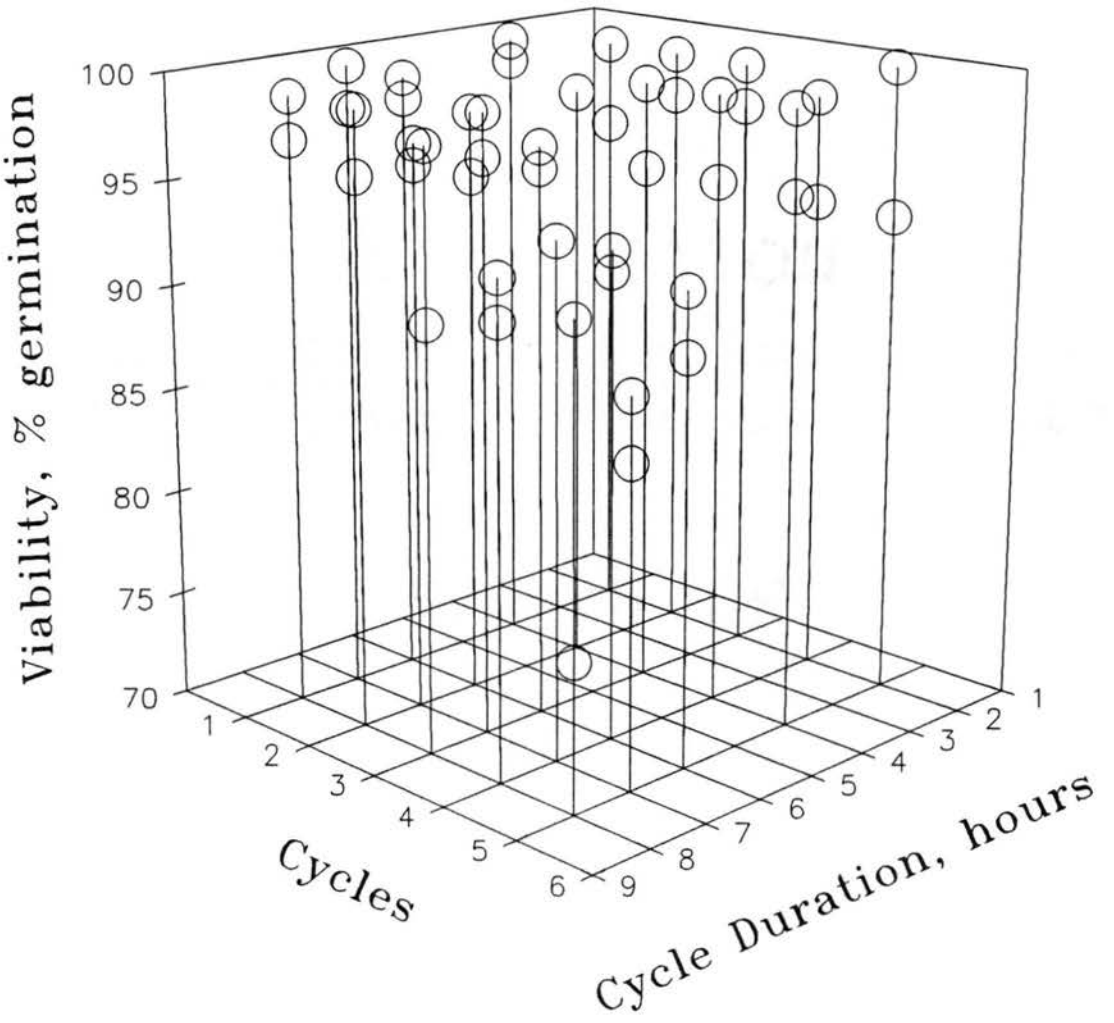

Viability, % germination
100
95
90
85
80
75
70
1
2
3
4
5
6
Cycles
1
2
3
4
5
6
7
8
9
Cycle Duration, hours

Figure 13. Primary data from which the response surface for relative vigor as a function of number of cycles and cycle duration was generated. Two replications were combined, providing n = 50 observations.

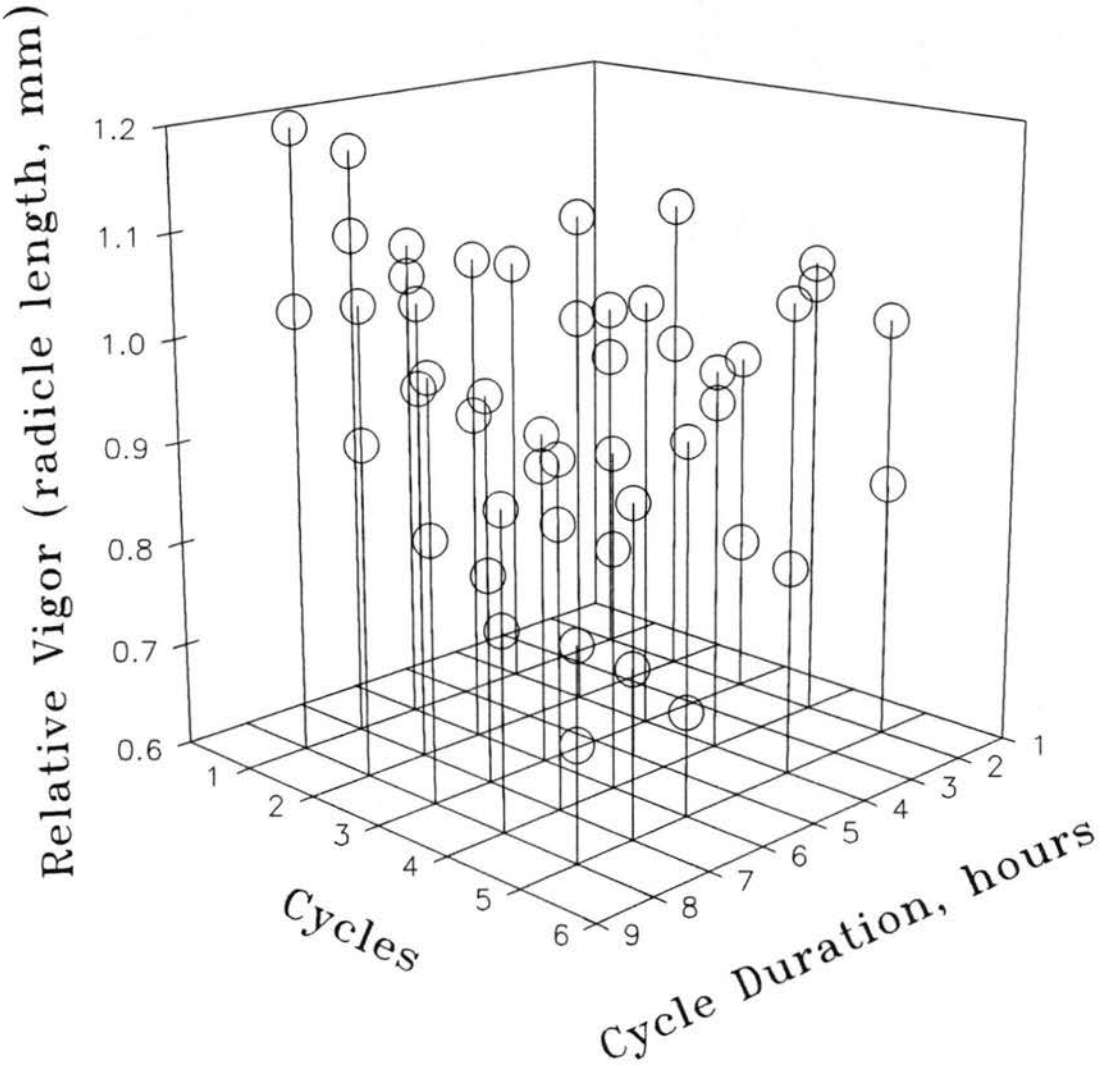

Relative Vigor (radicle length, mm)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
1
2
3
4
5
6
Cycles
1
2
3
4
5
6
7
8
9
Cycle Duration, hours

Table 3. Mean μAmp values determined at the end of each Cycle by Cycle Duration for replication two of the maize viability and vigor loss experiment.

Cycles	Cycle Duration, hours			
	2	4	6	8
1	34	45	43	52
2	9	12	15	15
3	7	12	15	17
4	7	12	18	18
5	10	15	15	20

nebulous yellow. Where the chalazal region had been prominent and noticeably dark before soaking had begun, it virtually disappeared after the soak was completed. The color was never regained and only faded more with subsequent soaking cycles. Leaching represents the loss of needed substances, such as inorganic ions, sugars, enzymes, nucleosides and nucleotides, fatty acids, carbohydrates, proteins, amino acids and nucleic acids as well as the loss of compartmentalization (Woodstock, 1988). The amount of potassium leakage correlates well with an increase in electroconductivity (personal communication with Dr. Sharon Sowa at NSSL). It could be that these compounds are responsible for the loss of color observed. Another possible explanation is that the membranes which have been hydrated are in functional form which resulted in the color change.

Models and Response

The best models, chosen from BMDP multiple regression analysis with consideration of bias and random error (using Cp versus p plots), are presented as response surfaces. Figure 14 represents the response surface for viability which was generated from the best model for viability, Table 4. Observation of Figure 14 reveals that four cycles of five hour soaks each caused only an 8% drop in viability, from 98% to 90% germination. Figure 15 represents the response surface generated from the best model for relative vigor, Table 5. The same four cycles, each of a five hour soak time, caused a 20% drop in relative vigor, Figure 15.

Figure 14. The best subset for viability, $Y_{VIA} = 99.1 - 0.0609(CD*C^2)$, $r^2 = 0.62$, presented as a response surface was chosen from a 2 factor, k = 9, 3rd order initial model.

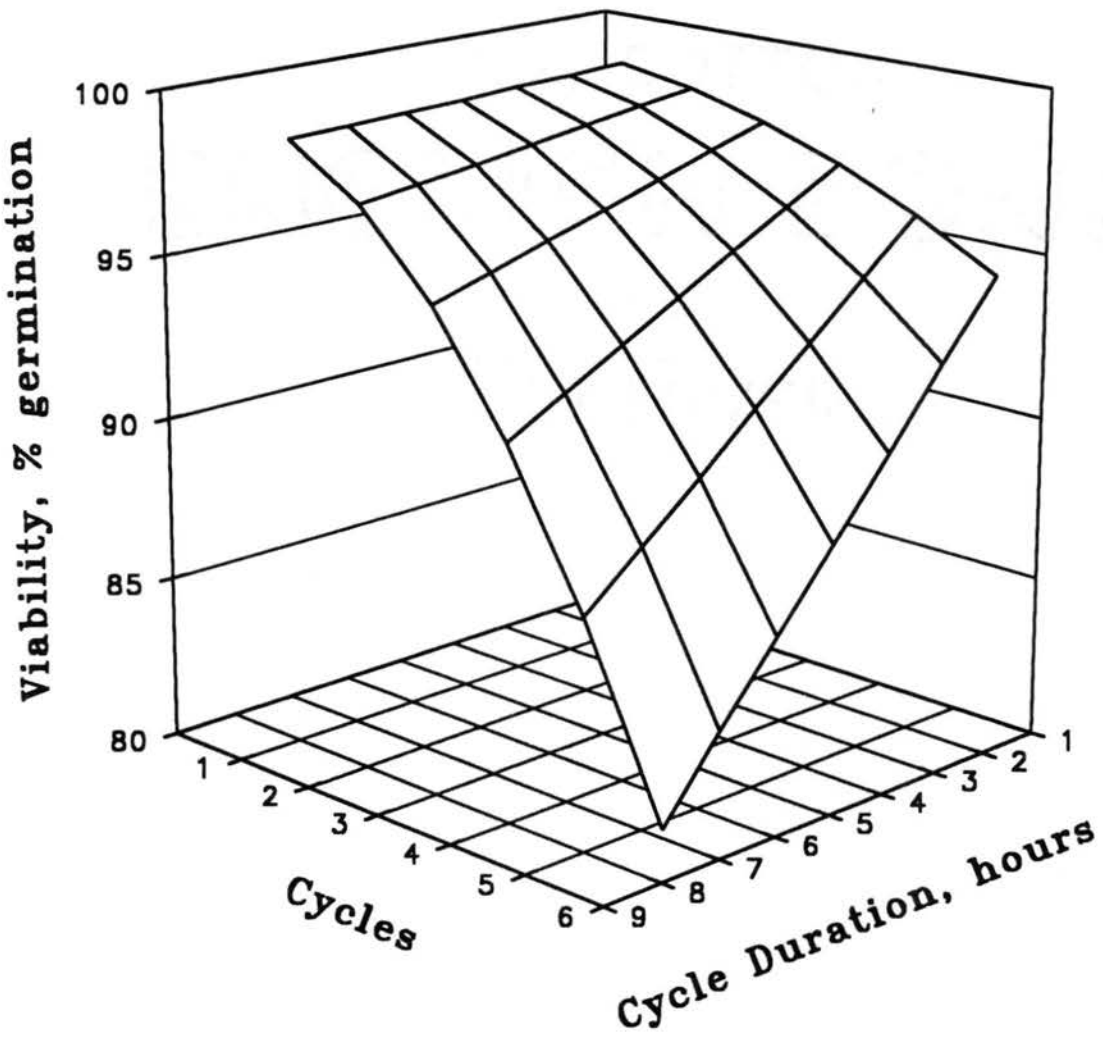

Viability, % germination
100
95
90
85
80
1
2
3
4
5
6
Cycles
9
8
7
6
5
4
3
2
1
Cycle Duration, hours

Table 4. Regression Statistics for Viability Loss Model, n = 50.

Variable Name	Regression Coefficients	T-Statistic	2 Tail Significance	Contribution to R^2
Intercept	99.1364	177.41	0.000	
CD * C^2	-0.0609	-8.86	0.000	0.62

$R^2 = 0.62$
$R_a^2 = 0.61$

Figure 15. The best subset for relative vigor, $Y_{RV} = 0.994 + 0.0229(CD) - 0.0101(CD*C)$, $R^2 = 0.52$, presented as a response surface was chosen from a 2 factor, k = 9, 3rd order initial model.

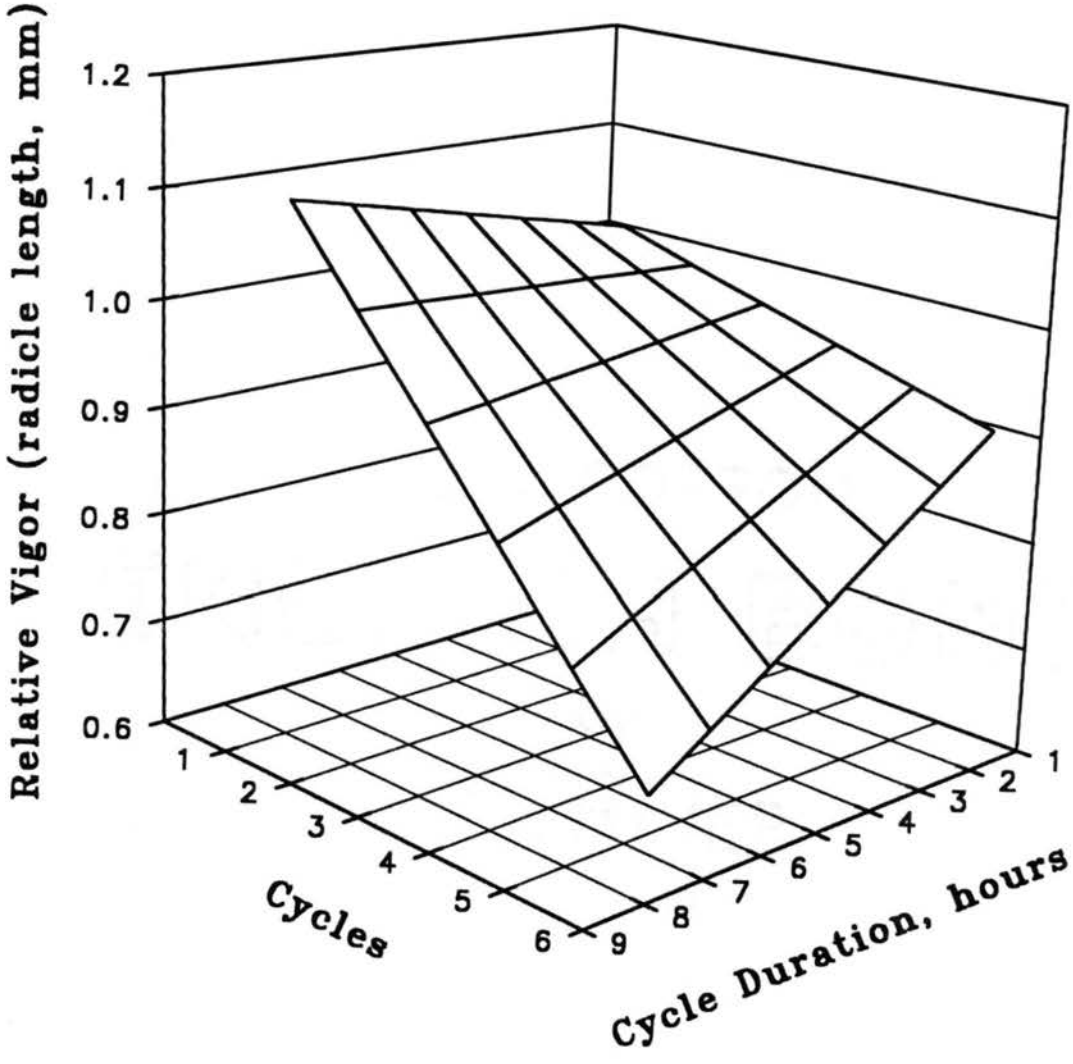

Relative Vigor (radicle length, mm)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
1
2
3
4
5
6
Cycles
9
8
7
6
5
4
3
2
1
Cycle Duration, hours

Table 5. Regression Statistics for Relative Vigor Loss Model, n = 50.

Variable Name	Regression Coefficient	T-Statistic	2 Tail Significance	Contribution to R^2
Intercept	0.9936	30.89	0.000	
CD	0.0229	3.26	0.002	0.11
CD * C	-0.0101	-6.96	0.000	0.50

$R^2 = 0.52$
$R_a^2 = 0.50$

Factorial ANOVA was performed to determine which, if either, factor, cycles or cycle duration, was more influential in the electroconductivity testing procedure, Table 6. Factorial ANOVA results revealed that cycles, constituting a hydration-dehydration treatment, were most influential for both viability and relative vigor at $P<0.01$. When the significance level was decreased to $P<0.05$, this remained true, although CD became an important factor for viability as well, Table 6. This can be seen in Figure 14 where the longer CDs cause a greater loss in viability and as the number of cycles increase the effect becomes more pronounced. Loss of cell membrane integrity could explain this phenomenon.

Visual inspection of the response surfaces led to an analysis of the effect of total soak time, considering both cycles and cycle duration, on both viability and relative vigor. Figures 16 and 17 show the viability and relative vigor response to the total number of hours each sample of 100 seeds was soaked for replications one and two, respectively. The combined effect of Cycles and Cycle Duration shows a steady, gradual decline for viability but a much steeper decline in relative vigor, indicating that vigor is more sensitive than viability. The same combined effect shows an initially increased relative vigor, compared to unsoaked controls, then a decline after about ten hours of soaking, a priming effect whereby the soaked seeds are invigorated could explain this. One effect of hydration treatments is to restore some of the seeds' ability to reorganize and repair membranes (Villiers and Edgcumbe, 1975; Nath *et al.*, 1991). The invigoration, or priming effect, observed has been shown to occur by hydration-dehydration treatments (Pammenter, 1974; Woodstock, 1988; Younis *et al.*, 1991).

Table 6. ANOVA - Influence of Replication, Dryback Cycles and Cycle Duration on Viability and Relative Vigor Response of Corn Seed.

Source	d.f.	Viability MS	Relative Vigor MS
Replication	1	11.52	0.003
Cycles	4	87.57**	0.072**
Cycle Duration	4	33.07*	0.012
C * CD	16	12.12	0.008
Error	24	9.77	0.009

** Significant at $P < 0.01$.
* Significant at $P < 0.05$.

Figure 16. Combined effect of number of Cycles and Cycle Duration (number of cycles X cycle duration hours) on viability and relative vigor. The correlation coefficients are significant at $P<0.01$ for viability and relative vigor. n = 25 data sets of 100 maize seeds, the first of two replications.

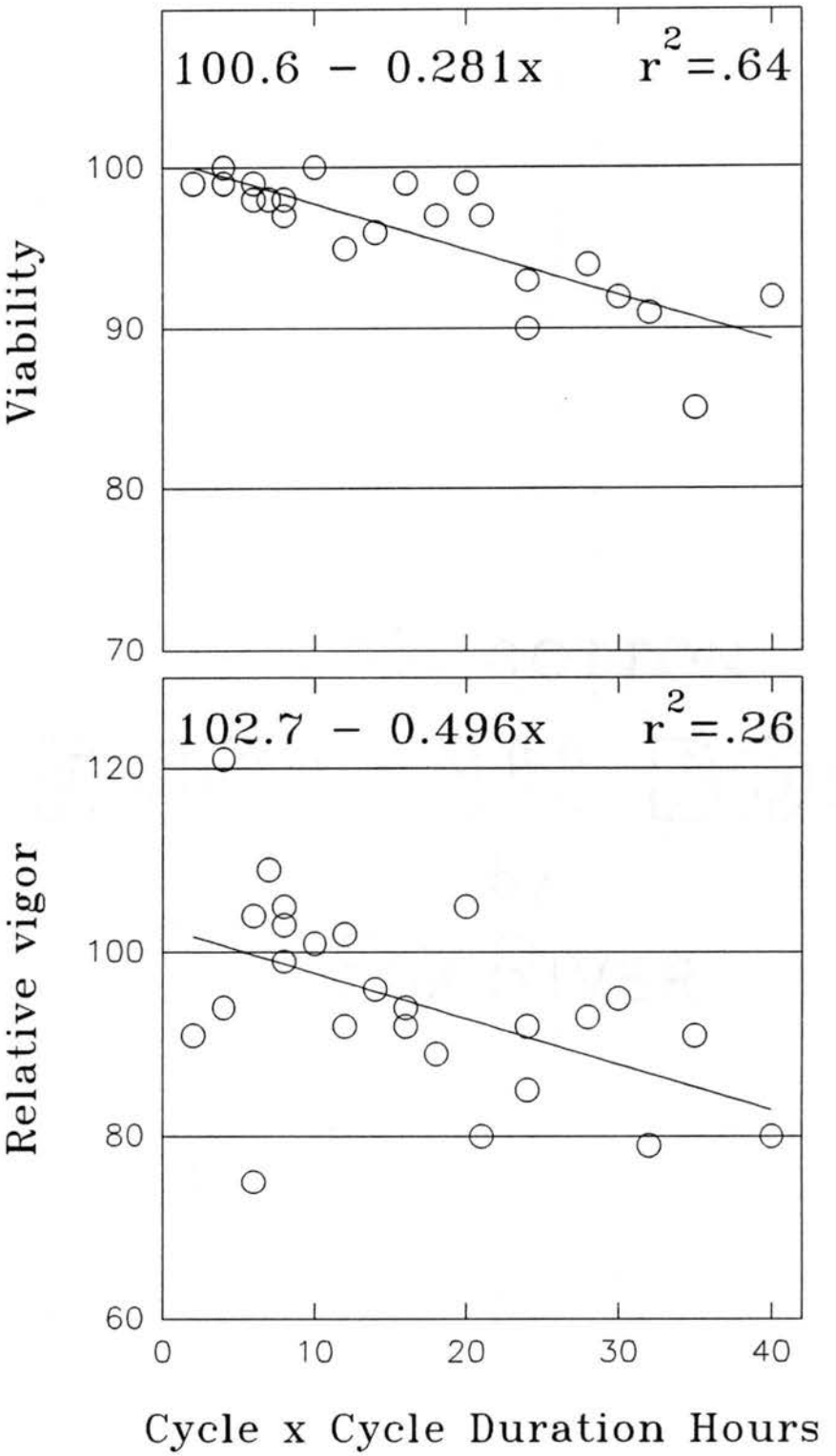
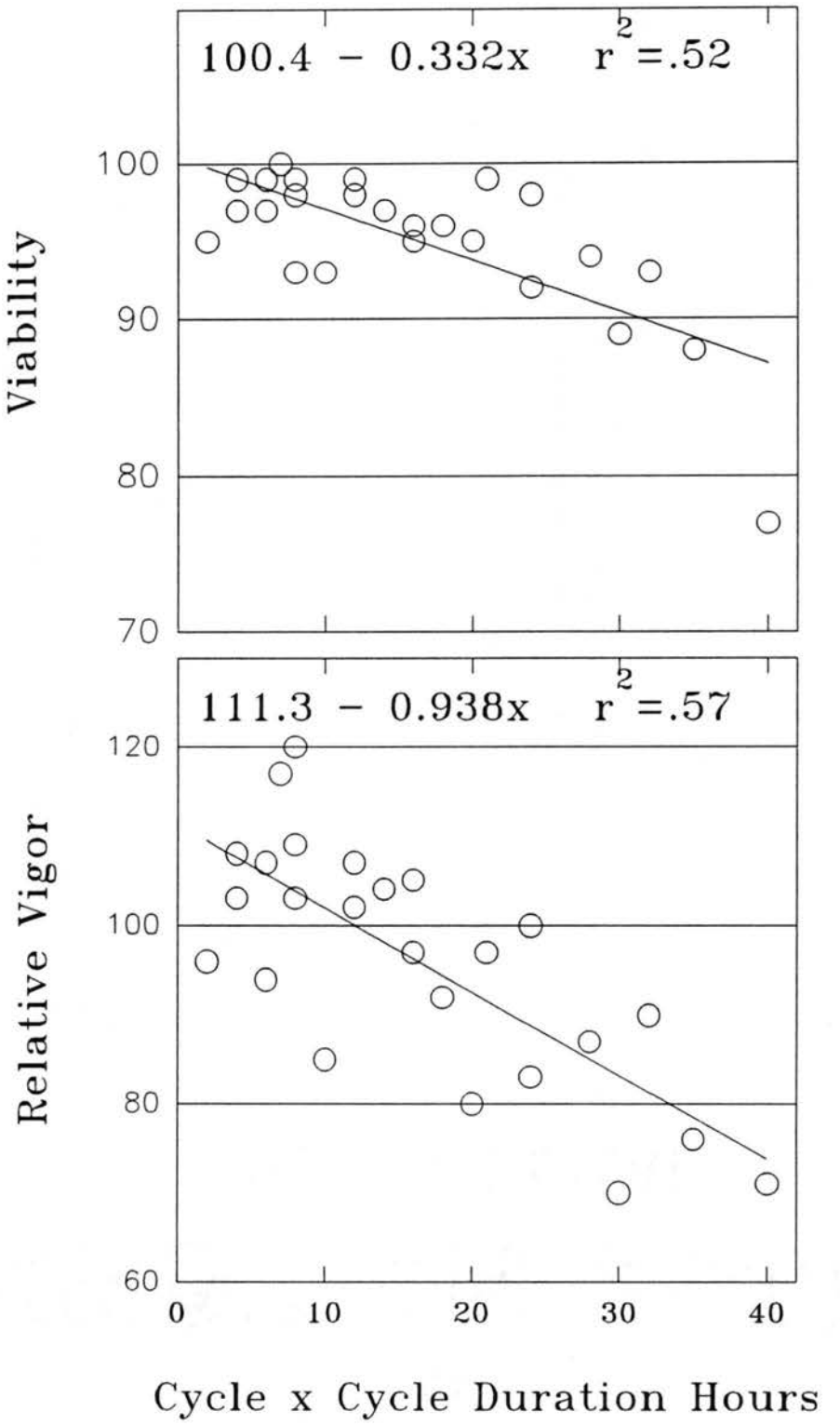

100.6 - 0.281x
r²=.64
Viability
100
90
80
70
102.7 - 0.496x
r²=.26
Relative vigor
120
100
80
60
0
10
20
30
40
Cycle x Cycle Duration Hours

Figure 17. Combined effect of number of Cycles and Cycle Duration (number of cycles X cycle duration hours) on viability and relative vigor. The correlation coefficients are significant at $P<0.01$ for viability and relative vigor. n = 25 data sets of 100 maize seeds, the second of two replications.


100.4 - 0.332x r² =.52
Viability
100
90
80
70
111.3 - 0.938x r² =.57
Relative Vigor
120
100
80
60
0
10
20
30
40
Cycle x Cycle Duration Hours

SUMMARY AND CONCLUSIONS

Electroconductivity Tests

Soak Time

The soak period can effectively be reduced to six hours for maize seed with sufficient electroconductivity data to predict the quality of the seed lot.

Seed Aging

The accelerated aging method developed by Delouche and Baskin (1973) and improved many times, affords the researcher the ability to obtain many levels of viability. The method developed by Vertucci and Roos (1990) did not perform as expected.

Measures of Maize Seed Quality

Electrolyte leakage appears to correlate well with maize seed quality. Internal slope, the median current value and the mean current value, μAmps, were the best indicators of seed quality; however Internal Slope has the rationale that its reciprocal measures the within sample variability (Moore *et al.*, 1988), unlike the other measures. The Initial Leach Rate and the Average Absolute Leach Rate showed no correlation with viability or relative vigor. The ILR did not predict seed quality because it is only a theoretical slope or maximum rate at the zero soak period. Ideally this index estimates the most rapid rate of leakage which occurs

upon first contact with free water, but this stage of leakage is extremely variable from seed to seed. ILR may estimate seed quality on a per seed basis, however it does not predict seed quality on a sample of 100 seed leachate conductivity values. The AALR does not predict seed quality because it is the integral mean of leakage rates over the entire soak period, taking into account both the initially rapid phase and the slower gradual phase whereby an asymptote is approached. These two phases of leakage would seem to be fundamentally different physiologically. Relative vigor was not predicted as accurately as viability, possibly because the correction in terms of the unsoaked controls was not as accurate as simply using the radicle length in millimeters. Non-aged and/or naturally aged seed should be tested to determine whether this is true. Continuous agitation of the soaking tray during testing would possibly improve the accuracy of the electroconductivity test procedure.

Viability and Vigor Loss

About 45% of the readily leachable electrolytes came out of the seeds during the first cycle of hydration, during which time the cellular membranes may have recovered selective permeability. Seeds undergoing subsequent cycles did not leach as many electrolytes, possibly because all of the readily leachable electrolytes leached during the first soak cycle. Also during the first soak cycle, the membranes recovered selective permeability and did not entirely lose this capacity upon dehydration, requiring less time to regain selective permeability and therefore losing less electrolytes with each successive soak. Four cycles of five hour soaks resulted

in only an 8% loss of viability and a 20% loss of relative vigor. Factorial ANOVA revealed that the number of cycles is more deleterious to the seed than the duration of the cycle, probably due to the loss of cellular components. Lastly, there is a priming effect, invigoration, with regard to relative vigor observed for seeds soaked approximately ten hours. Storage periods between soak cycles should be added in any future simulation of electroconductivity testing.

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APPENDIX

Figure 18. Linear regressions, with 95% confidence bands, of viability and relative vigor on radicle length for 10 replications each of 100 aged maize seeds. Both correlation coefficients were significant at $P < 0.05$.

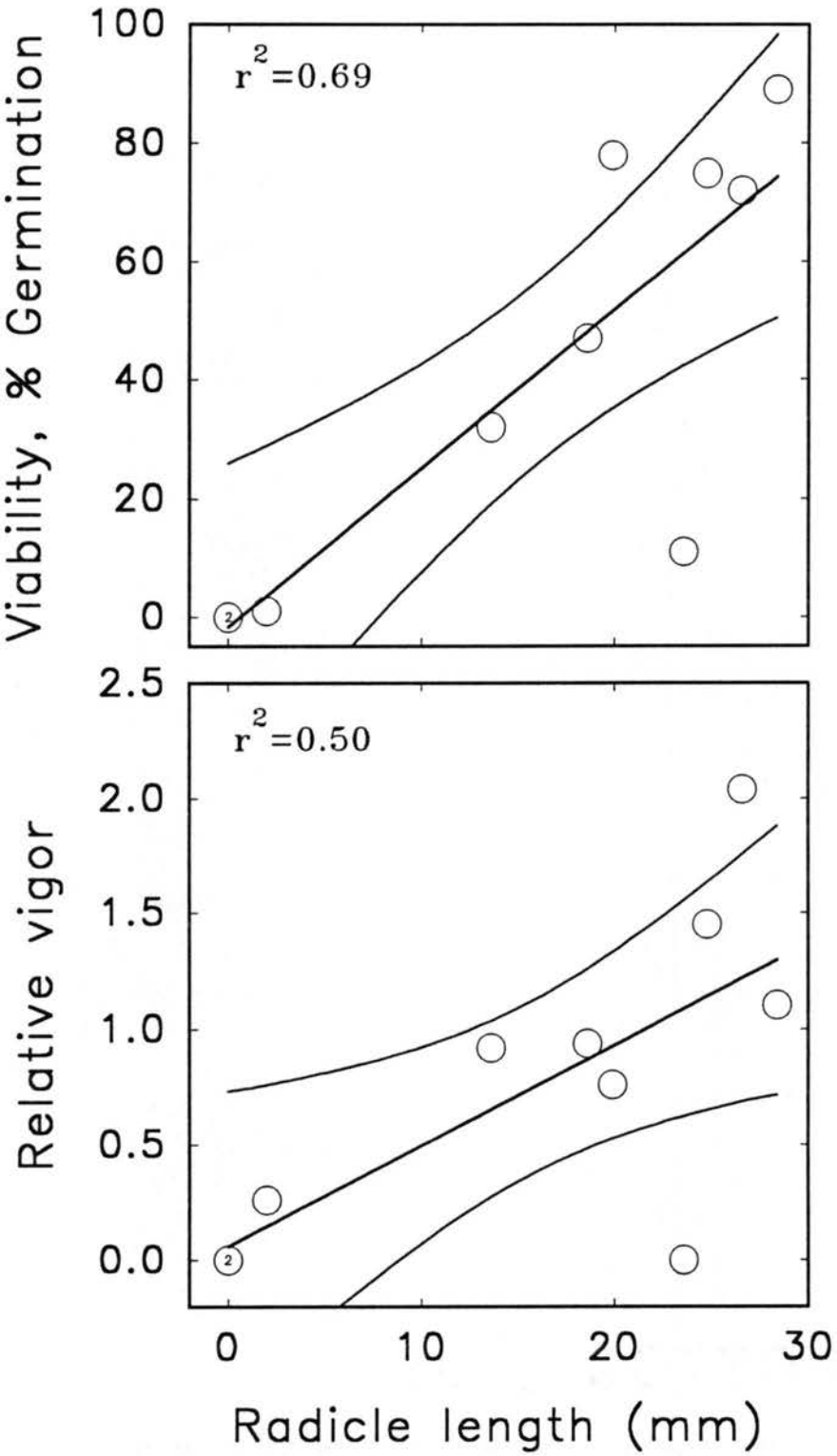

Viability, % Germination
100
80
60
40
20
0
r²=0.69
Relative vigor
2.5
2.0
1.5
1.0
0.5
0.0
r²=0.50
0
10
20
30
Radicle length (mm)

Figure 19. Two maize seed lots 88-1d and 88-2i, were aged using Method A. Each line represents the rectangular hyperbolic function fitted to 10 replications of 100 aged maize seeds. The individual data points shown with 95% confidence intervals represent an average of the 10 observed current values, μAmps, seen at 5 hours imbibition, for 88-1d, open circle, and 88-2i, closed circle, maize seed lots.

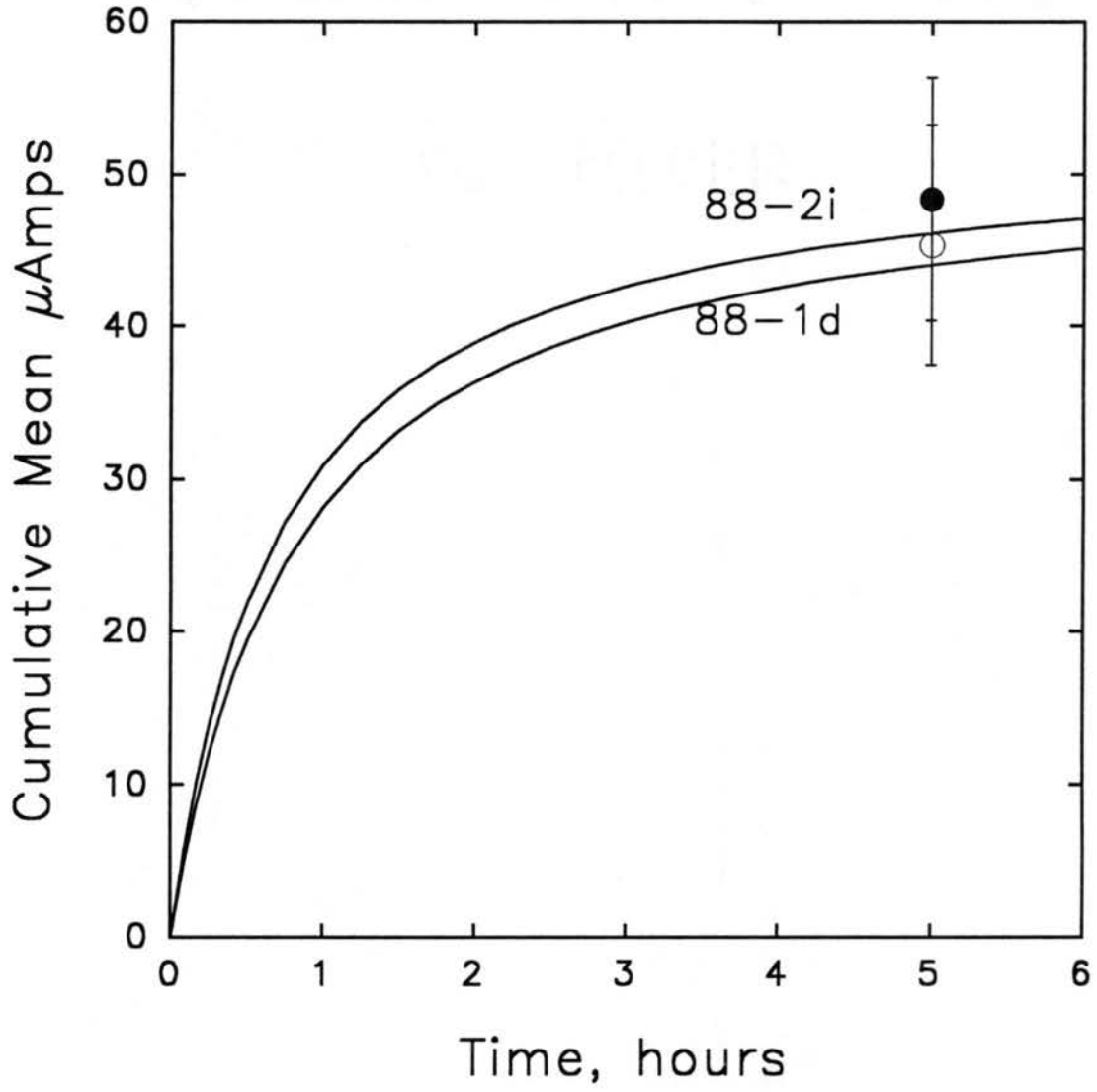
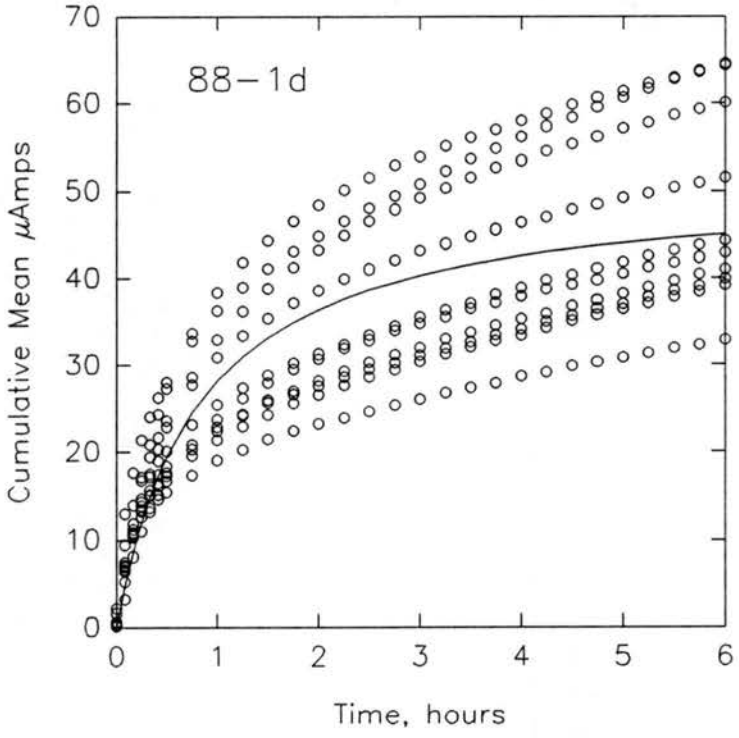

Cumulative Mean μAmps
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Time, hours
88−2i
88−1d

Figure 20. The rectangular hypebolic function fitted to ten replications of 100 Method A aged maize seeds for two seed lots, 88-1d and 88-2i.


88–1d
Cumulative Mean μAmps
Time, hours
0
10
20
30
40
50
60
70
0
1
2
3
4
5
6

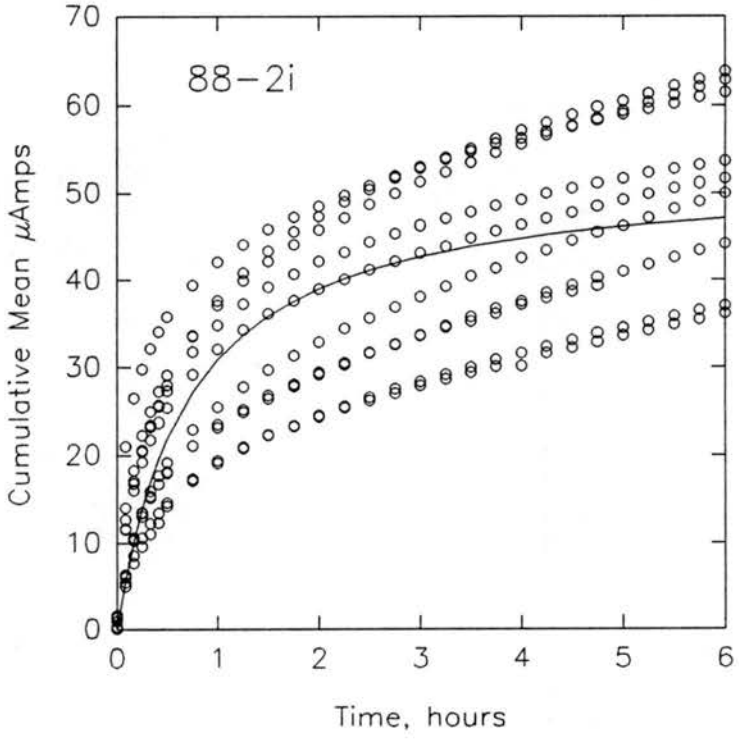

88–2i
Cumulative Mean μAmps
Time, hours
0
10
20
30
40
50
60
70
0
1
2
3
4
5
6

Figure 21. The rectangular hyperbolic function fitted to 17 replications each of 100 Method B aged maize seeds for 238 days, lot 88-1d.

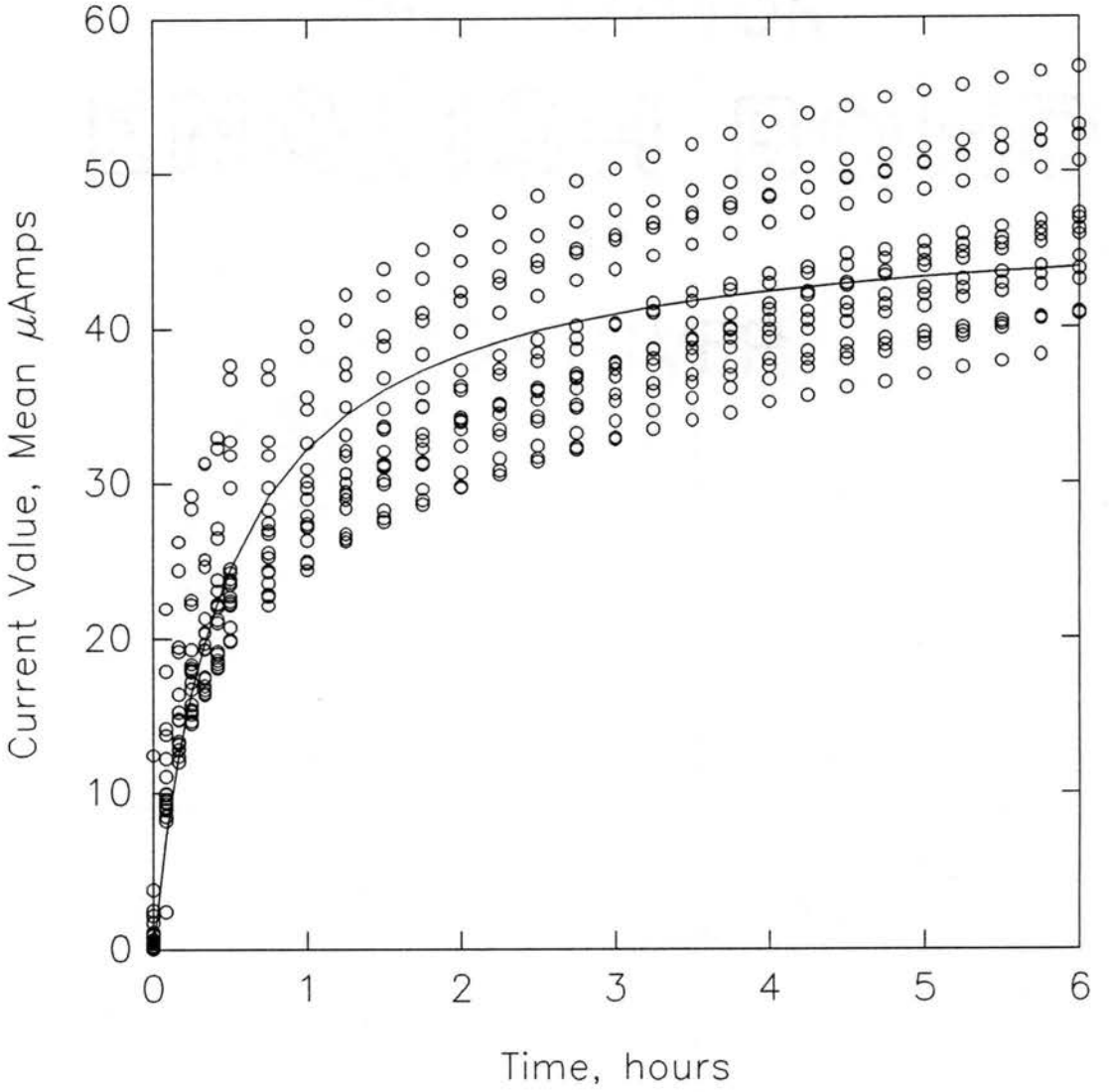

Current Value, Mean μAmps
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Time, hours

Figure 22. The rectangular hyperbolic function fitted to 17 replications each of 100 Method B aged maize seeds for 484 days, lot 88-1d.

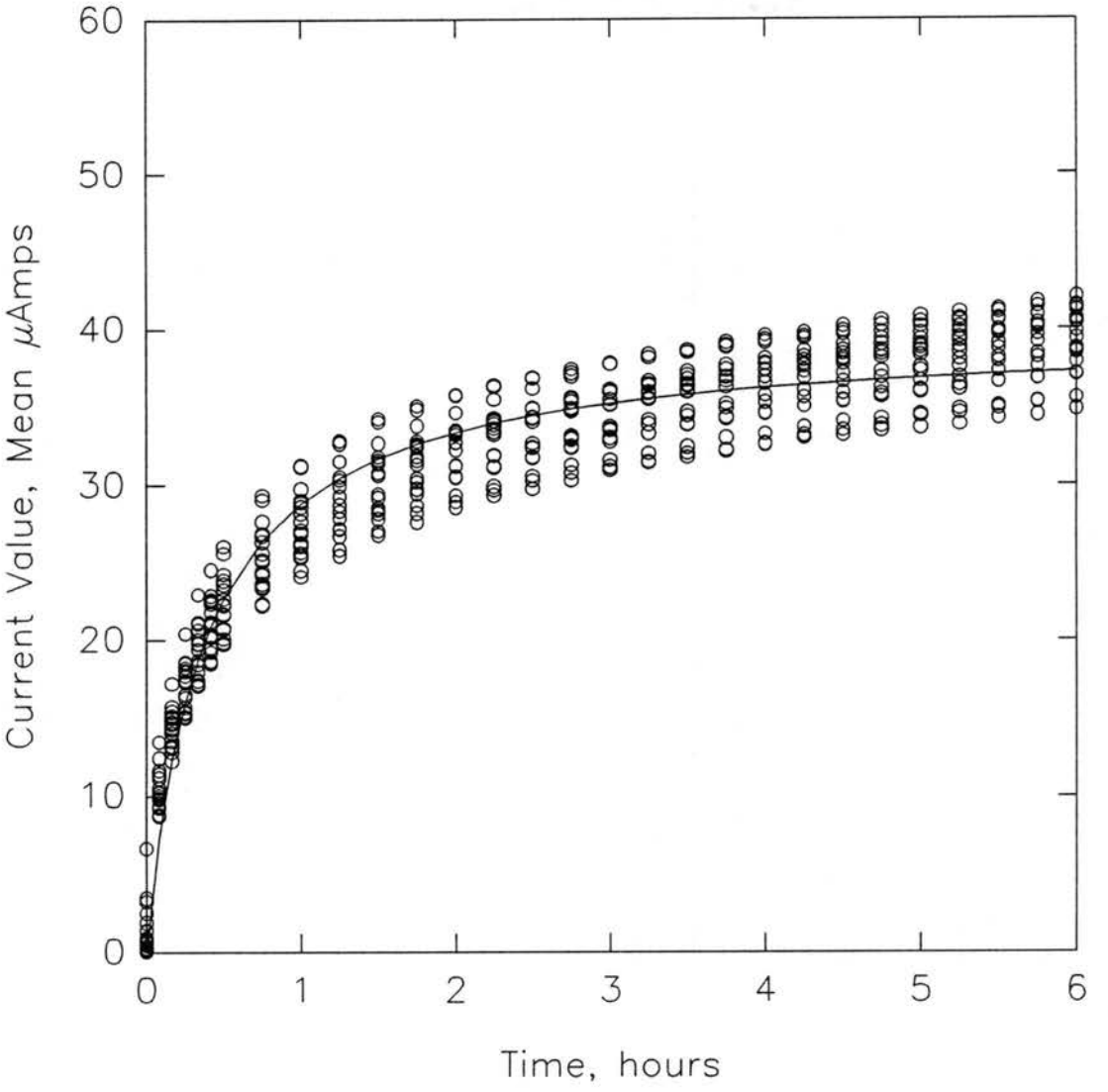

Current Value, Mean μAmps
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Time, hours

Table 7. Moisture contents (dry weight basis) of 10 seeds per sample after equilibration over saturated NaCl solution for 16 and 20 days, for seed lots 88-2i and 88-1d, respectively, prior to Method A aging.

Run		Moisture Content	% Germination
88-1d	1	14.9	89
	2	15.8	32
	3	16.0	78
	4	15.8	11
	5	16.1	0
88-2i	1	14.9	1
	2	15.4	75
	3	15.7	0
	4	15.0	72
	5	16.6	47

Table 8. Viability and Relative Vigor in response to aging. The break after replication J for Method A denotes where the first aging experiment was completed and the second began.

Rep	Method A		Method B, 238 days		Method B, 484 days	
	% Germ	Relative Vigor	% Germ	Relative Vigor	% Germ	Relative Vigor
A	89	1.10	98	1.00	92	1.06
B	32	0.92	99	1.07	84	0.82
C	78	0.76	96	1.09	90	1.08
D	11	0.00	98	1.06	90	1.22
E	0	0.00	96	1.18	86	1.14
F	1	0.26	96	0.85	84	0.58
G	75	1.45	98	1.37	84	0.80
H	0	0.00	97	1.06	84	1.89
I	72	2.04	91	1.23	75	1.18
J	47	0.94	91	0.85	86	0.94
K	9	0.46	94	1.10	88	0.97
L	8	0.00	98	1.04	73	1.60
M	14	0.00	89	1.24	77	1.19
N	7	0.00	98	1.49	84	0.93
O	5	1.43	98	1.11	69	1.34
P	0	0.00	93	0.92	61	1.81
Q	1	0.00	97	1.17	73	1.18
R	2	0.00	--	--	--	--
S	0	0.00	--	--	--	--
T	1	0.60	--	--	--	--

Table 9. Linear regressions of 1/μAmps on 1/time were performed to obtain the positive slopes of the regression line as a possible indicator of seed quality. See Table 7 for quality measurements.

Replication	Slope of Linear Regression of 1/μAmps on 1/time. ($* 10^{-3}$)		
	Method A	Method B 238 days	Method B 484 days
A	22	3	6
B	14	2	7
C	8	5	6
D	12	5	6
E	6	8	8
F	5	6	8
G	6	7	5
H	6	7	9
I	2	7	6
J	3	5	6
K	10	8	5
L	11	7	7
M	10	7	7
N	10	8	6
O	9	6	6
P	15	8	7
Q	11	7	7
R	14	--	--
S	11	--	--
T	12	--	--

Table 10. Viability, % germination, data from simulation of successive seed tests on maize, Pioneer 3541, for replication 2.

Cycles	Cycle Duration, hours				
	2	4	6	7	8
1	95	99	99	100	99
2	97	98	98	97	96
3	97	99	96	99	98
4	93	95	92	94	93
5	93	95	89	88	77

Table 11. Relative vigor data from simulation of successive seed tests on maize, Pioneer 3541, for replication 2.

Cycles	Cycle Duration, hours				
	2	4	6	7	8
1	0.96	1.03	1.07	1.17	1.20
2	1.08	1.09	1.07	1.04	1.05
3	0.94	1.02	0.92	0.97	1.00
4	1.03	0.97	0.83	0.87	0.90
5	0.85	0.80	0.70	0.76	0.71

Table 12. Imbibed moisture content, percent based on fresh weight, determined at the end of each Cycle by Cycle Duration treatment for replication 2 of the maize viability and vigor loss experiment.

Cycles	Cycle Duration, hours				
	2	4	6	7	8
1	13	15	22	21	23
2	19	18	27	31	28
3	24	35	33	34	36
4	32	26	38	43	36
5	33	35	33	30	40