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Solute Loss from Deteriorated Cottonseed: Relationship Between Deterioration, Seed Moisture, and Solute Loss[1]

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ABSTRACT

Loss of electrolytes from cottonseed (*Gossypium hirsutum* L.) soaked in water was influenced by factors in the processing history of the seeds, such as delinting procedure and post-delinting washing. Measurements of electrolyte loss were most reproducible if they determined a change during an interval (15 to 45 min) not inclusive of zero time. Cottonseed (*G. hirsutum* 'Deltapine 16') that deteriorated under controlled conditions of 100% relative humidity and 35 to 50 C was reduced in viability and exhibited increased rates of solute leakage and moisture contents. Seeds that deteriorated under conditions in which their moisture contents were held at 20% showed a slight increase in solute loss upon deterioration, but the rates of solute loss were not significantly correlated with viability losses. Experiments with both viable and heat-killed seeds revealed that the increases in solute loss were proportional to degrees of seed hydration. Solute loss from heat-killed seeds was greater than that from viable seeds at equivalent moisture contents, indicating that embryo tissue viability influenced solute leakage. However, increased solute losses due to differences in seed moisture were of greater magnitude than those due to changes in seed viability. Rate of electrolyte loss is therefore not a valid measure of cottonseed deterioration.

Additional index words: ***Gossypium hirsutum*** **L., Permeability, Electrolyte loss, Accelerated aging.**

DETERIORATION of cottonseed (*Gossypium hirsutum* L.) is accelerated by high seed moisture and high temperature (1). Presley (7) first described the rapid, controlled deterioration of cottonseeds by incubation at 50 C and 100% relative humidity. He observed that deterioration was correlated with increased electrical conductance of seed leachates and suggested that electrolyte loss might provide a method of evaluating seed deterioration. Presley and Schnathorst (8) and Schnathorst and Presley (9) reported that carbohydrates and amino acids were among the substances present in higher concentrations in leachates from deteriorated seeds than in leachates from viable seeds. Bird and Reyes (2, 3) reported findings opposite from the previous ones, stating that concentrations of electrolytes (electrical conductance) and carbohydrates were lower in leachates from deteriorated seeds than in leachates from viable seeds. My investigations were undertaken to resolve these conflicting findings and to evaluate solute concentrations in seed leachates as a measure of the viability of cottonseed.

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[3] Mention of specific equipment or commercial varieties does not constitute endorsement by the USDA over similar equipment or other varieties not mentioned.

MATERIALS AND METHODS

Cottonseed of *G. hirsutum* 'Deltapine 16' used in these experiments were acid-delinted and density graded. Seeds were washed six times in distilled water to remove salts remaining on or in the seed coats as a result of delinting. These prewashings consisted of stirring seeds for 1 min in three volumes (w/v) of distilled water. Seeds were then air-dried at room temperature (22 C), until they contained between 7 and 8% moisture and stored in sealed containers at 6 C. Visibly damaged and immature seeds were removed.

Seeds were deteriorated by being incubated in moist atmospheres in hermetically sealed 0.946 liter (1 qt) jars at 30 to 50 C. Samples of 50 seeds were placed in cheesecloth bags, and four to six bags were suspended by rubber bands from a hook in the center of the jar lid. Direct contact of the bags with condensed moisture on the surfaces of the containers was thus avoided.

Two incubation procedures were utilized. Procedure 1 consisted of placing 25 ml of water in the bottom of each jar along with the seed samples. This produced 100% relative humidity, which enabled the seeds to imbibe throughout the incubation period. In procedure 2, seeds were preincubated as in procedure 1 at 21 C for 48 hours. This resulted in imbibition of the seeds to 20% moisture (wet-weight basis, $\pm$ 0.4% moisture). Seeds were then transferred to jars containing the small amount of water (e.g. 0.037 ml for 35 C, 0.079 ml for 50 C) required to saturate the atmosphere of the jar without leaving any residual liquid. In this manner, seeds were held at constant 20% moisture throughout the deterioration treatment.

Following deterioration, seeds were removed from the cloth bags, weighed immediately, and immersed in 25 ml of distilled water in 30 ml beakers. Duplicate samples were weighed, placed in a drying oven at 105 C for 48 hours, and reweighed to determine moisture content. Electrical conductance of the seed leachates was measured with a Yellow Springs Instrument Co., Model 31, conductivity bridge equipped with a Model 3403 cell with a cell constant of 1.0. Conductance was determined 15 and 45 min after immersion of seeds in water. Data shown represent the difference between these readings per-gram dry weight of seed.

Concentrations of specific leachate components were measured in solutions obtained when seeds were soaked in distilled water for 60 min and are expressed as $\mu g \times g$ (dry wt) of seed^{-1}. Immediately before analysis, solutions were filtered through Millipore filters with a pore size of 0.45 μ. Ca, Mg, K, and Na concentrations were determined on a Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer. Total carbohydrate was measured by the method of Dubois et al. (5); maltose was used as the standard.

Failure of seeds to germinate was used as the primary criterion of deterioration. To effect germination seeds were wrapped in 10$\times$46 cm strips of moistened Whatmann No. 3 filter paper and incubated at 30 C for 48 hours. Seeds which failed to produce a radicle 3 mm in length were considered non-germinated.

Data reported, other than regression analyses, are the average of five replicate samples of 50 seeds each. Deviations from materials and methods described in this section are noted in the discussions of specific experiments.

RESULTS AND DISCUSSION

Processing of cottonseed involves various steps which can result in deposition of solutes on or in seed coats. The most notable of these is delinting of seeds with sulfuric acid. Background levels of solutes should be reduced to prevent masking any effects of treatments. Figure 1 illustrates the changes in leachate conductance obtained with various numbers of washings of seed. Most extraneous ionic material was removed by two washings. The effect of various numbers of washings on leachates obtained during 15 or 45 min was essentially the same.

The expression of conductance as the difference between the 15- and 45-min readings represents a departure from the method employed by others (2, 3, 7), who measured conductance only after 15 min or less. When seeds were deteriorated by procedure 1 and conductance changes were measured at 15 min, the results were similar to those of Presley (7). Frequently, however, samples were encountered which differed greatly from other replicate samples. When conductance of the samples was measured as the difference between 15- and 45-min readings, values for replicate samples were more uniform. Further, the slope of the difference line for 15 to 45 min was much less than that for either 15 or 45 min. Therefore, the effects of minor differences in the handling of individual samples on conductance are reduced in magnitude by using difference readings. Additional evidence of reproducibility of results obtained from 15 to 45 min readings is presented in Table 1. Observed differences between control and uniformly deteriorated seeds were relatively consistent, despite differences in cultivar, delinting procedure and prewashing. Readings taken only after 15 min for control samples, however, ranged from 17 to 147 μmhos/g of seed.

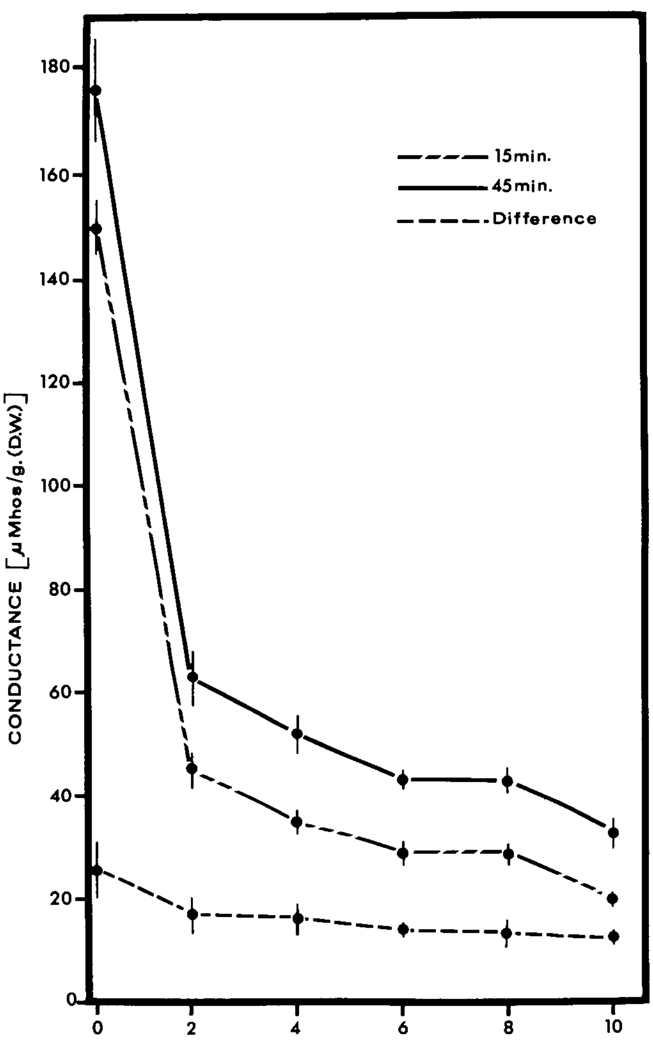

Fig. 1. Effect of washing seeds with distilled water on the subsequent conductance of seed leachates.

Except for a few highly different samples, I have found that proportional differences in the rate of electrolyte leakage between treatments persisted for at least 1 hour; the rates of electrolyte leakage always decreased with time.

Deterioration and Solute Loss

When seeds were deteriorated by procedure 1, a method similar to those used by others (2, 3, 7), the rate of electrolyte loss was greater in deteriorated than in control seeds. Percentages of germination and conductance of leachates were negatively correlated (Fig. 2), as reported by Presley (7). Increased leachate conductance as a result of deterioration is further substantiated by results in Table 1. Regardless of variety, delinting method, or prewashing treatment, conductance of leachates from deteriorated seeds was higher than that of leachates from controls.

Table 1. Electrolyte leakage from cottonseed as influenced by deterioration in a water-saturated atmosphere (7 days, 35 C), delinting procedure and prewashing.

Delinting procedure	Prewash*	Conductance			Germination		
		Control	Deter.	Diff.	Control	Deter.	Diff.
		μmhos			%		
Deltapine 16							
Machine†	-	20	30	10	91	61	30
	+	11	22	11	89	60	29
Acid‡	-	22	35	13	94	58	36
	+	21	28	7	96	64	32
Stoneville 213							
Machine†	-	25	36	11	93	48	45
	+	13	23	10	88	34	54
Acid†	-	26	37	11	99	70	29
	+	22	28	6	98	64	34
Acid‡	-	60	71	11	80	0	80
	+	52	65	13	74	0	74

* Prewashing consisted of two washings with distilled water. † Seeds produced in Mississippi. ‡ Seeds produced in Arizona.

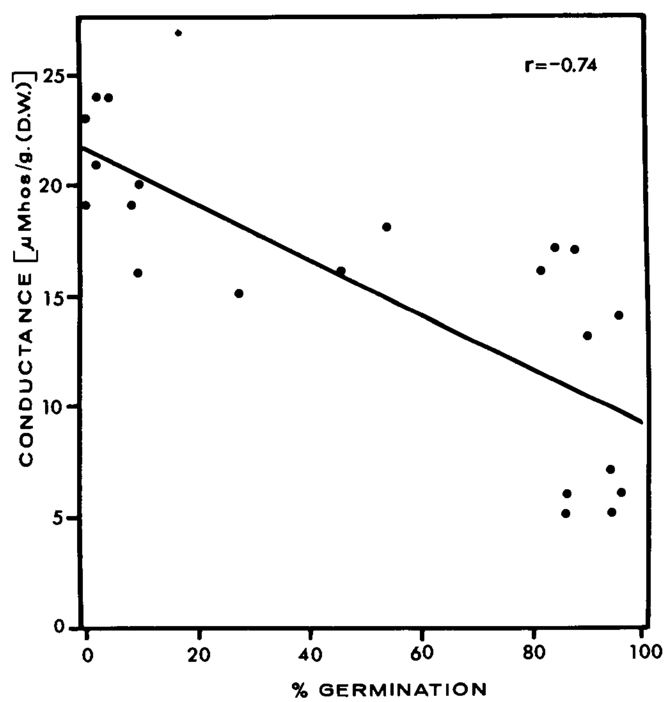

Fig. 2. Relationship between seed viability and electrical conductance of leachates, when seeds were deteriorated at 50 C, in a water-saturated atmosphere, for various periods of time.

In the above experiments seed moisture levels were not controlled, and results might have been influenced by changes in the moisture contents of seeds, as well as by deterioration. The experiments were repeated using deterioration at a constant 20% moisture (procedure 2), with incubation periods of 0 to 10 days and temperatures of 35, 40, 45, and 50 C. Results of the experiments at 35 and 50 C are presented in Fig. 3. Results from experiments at 40 and 45 C were similar to those at 35 and 50 C. At 50 C, conductance and carbohydrate concentration increased (Fig. 3A and B) whereas germination decreased (Fig. 3C). Values for conductance and carbohydrate concentration, however, did not differ significantly (LSD 5%) from those of the control until the fourth day, when none of the seeds germinated. At 35 C, results were highly erratic, and deteriorated seed did not differ significantly from the controls, except in germination, until the tenth day. Mold growth was visible on all but 50 C-deteriorated seeds, and probably influenced the conductance readings (Fig. 3A) and the carbohydrate concentrations (Fig. 3B), particularly at 35 C.

The concentration of K ions corresponded closely to the conductance of seed leachates. For example, the K concentration for seed incubated 5 days at 50 C was 212 μg/g, compared to 146 μg/g for controls. Sodium, calcium, and magnesium concentrations ranged between 49 and 59 μg/g, 2.5 and 2.9 μg/g, and 0.5 and

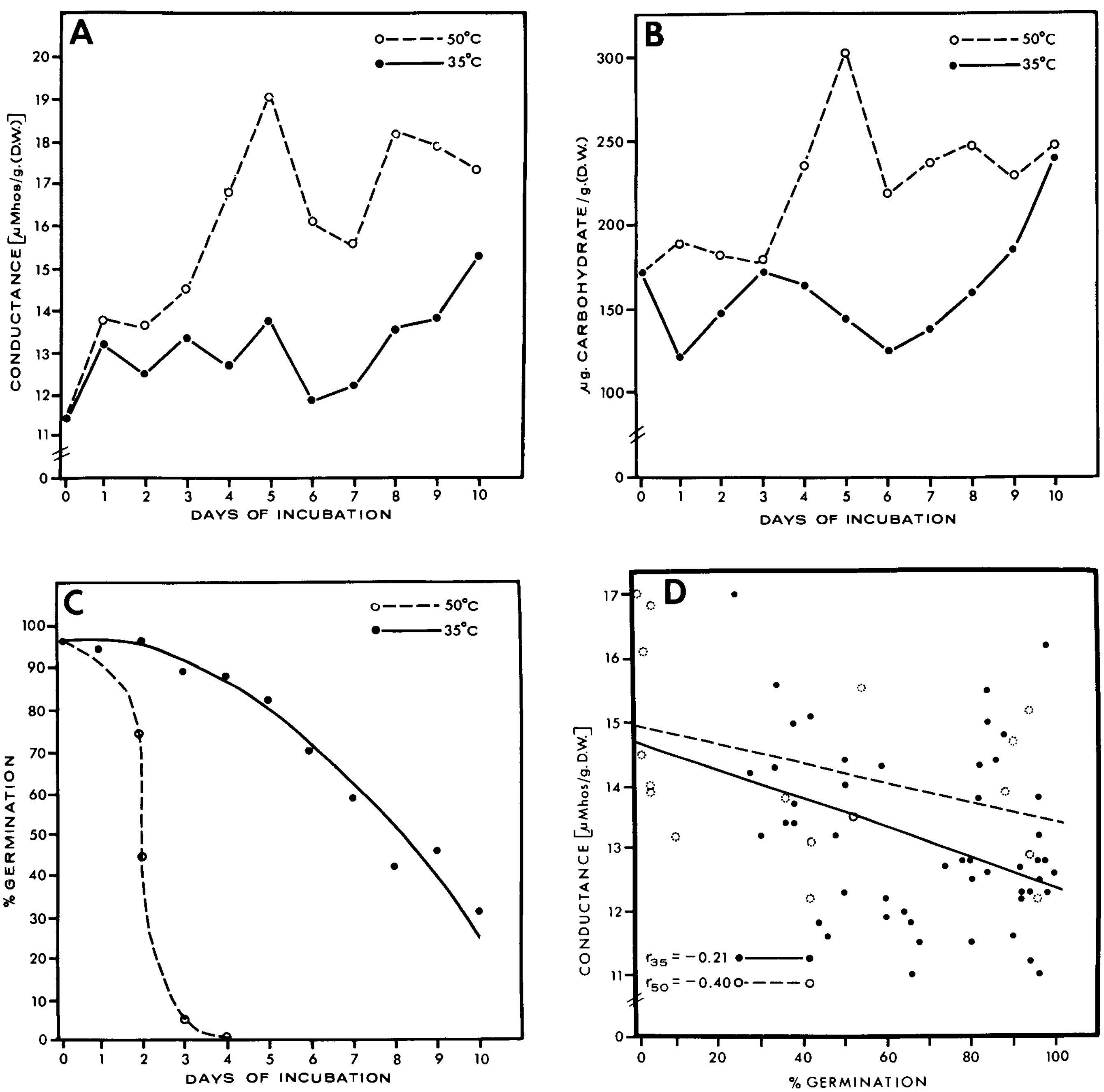

Fig. 3. Effects of deterioration for 0 to 10 days at 35 and 50 C with constant seed moisture. A, Conductance of seed leachates; B, carbohydrate concentrations in seed leachates; C, seed viability; D, relationship between seed viability and seed leachate conductance.

1.0 μg/g, respectively; there were no significant differences (5% level) between treatments.

Percentage of germination versus conductance for seeds deteriorated at 35 and 50 C is presented in Fig. 3D. Only the regression coefficient for the combined 35 and 50 C values ($r = 0.28$) was significant at the 5% level, and the difference of conductance between 100% and 0% germination was only 2.2 μmhos. This was less than the observed standard deviations within many of the replicated treatments. Thus, when seeds are deteriorated under conditions of constant seed moisture, the degree of deterioration has little effect on the loss of solutes from the seeds. This is particularly true within the range of deterioration which allows some seeds to retain viability.

Seed Moisture and Solute Loss

Because deterioration and solute loss were significantly correlated when seeds were deteriorated by procedure 1 but not by procedure 2, seed moisture might be a major controlling factor in solute loss. When percentages of seed moisture for viable seed treated by procedure 1 were plotted against conductance (Fig. 4), a significant (1% level) positive correlation ($r = +0.80$) was obtained. This suggests that seed moisture content at the end of the deterioration treatment determines the amount of electrolyte leached from the seed.

The data presented in Fig. 2 and 4 might lead to conflicting or confusing interpretations, because both percentage of germination and moisture content were related to conductance with similar regression coefficients, both significant at the 1% level.

To distinguish the influence of moisture content from that of seed viability on electrolyte leakage, it was necessary to reduce the viability of all seeds to zero, then vary the moisture content. Seeds killed in a forced air oven at 110 C for 48 hour were brought to various moisture levels by means of deterioration procedure 1. Moisture content was varied in two ways: a) seeds were incubated at 50 C for various periods of time ranging from 1 to 7 days (Fig. 4), and b) seeds were incubated for 48 hours at temperatures ranging from 6 to 60 C. Regression plots for the two experiments were similar, indicating that time and temperature of incubation had little effect on the results. The relationship between seed moisture and leachate conductance ($r = 0.94$) was highly significant at the 1% level in both experiments.

At all moisture levels, the amount of electrolyte leakage from heat-killed seeds was higher than that obtained from viable seeds. Although seed moisture is the major determinant of electrolyte concentration in seed leachates, seed viability has some influence.

Another experiment was done to determine if seed moisture content at the end of the deterioration period or at the time of determining leachate conductance was more critical in determining rate of electrolyte loss. Separate lots of seed were deteriorated at 35 C by procedures 1 and 2 for 0 to 14 days. Seeds were air-dried for several days to between 7 and 9% moisture before measurement of conductance. Electrolyte loss from deteriorated samples showed no significant increase over that from undeteriorated controls, until

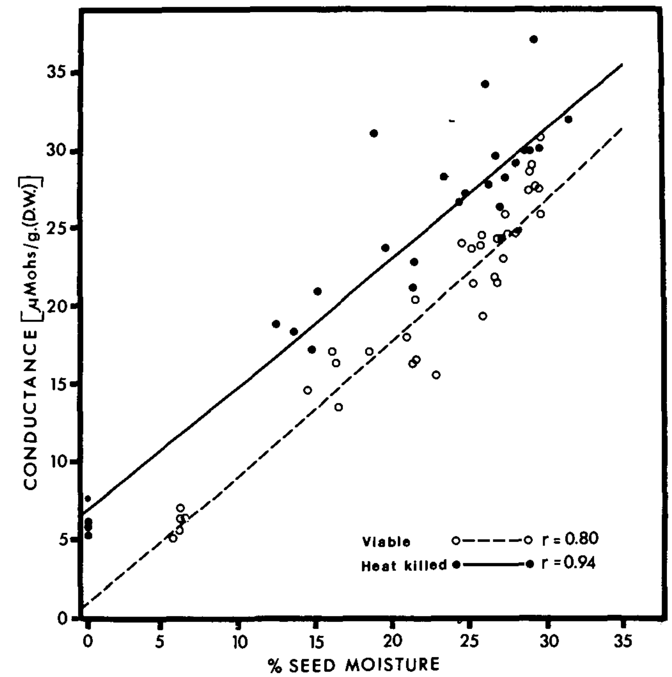

Fig. 4. Relationship between seed moisture content and conductance of seed leachates: viable (○ - - - ○) and heat-killed (● —— ●) seeds were deteriorated at 50 C, in a water-saturated atmosphere, for various periods of time.

less than 10% of the seeds were viable. This demonstrated that seed moisture content at the time of conductance measurement is critical in determining the rate of electrolyte loss from deteriorated seeds.

Electrical conductance of seed leachates was correlated with seed moisture under all conditions studied, but was correlated with seed viability only under conditions that allowed seeds to imbibe throughout the deterioration process. Thus, increased electrical conductance in seed leachates, associated with deterioration under conditions of high temperature and high humidity is primarily a measure of the state of seed hydration; less increase in conductance is caused by loss of differential membrane permeability in killed embryo tissues. Electrical conductance of leachates is, therefore, not a valid measure of cottonseed deterioration under conditions of high or variable seed moisture. These conclusions agree with those from previous findings on other species. Ching and Schoolcraft (4) found that increased concentration of solutes in leachates from *Trifolium incarnatum* and *Lolium perenne* was related to loss of vigor, but the magnitudes of increases were related to seed moisture. Hibbard and Miller (6) concluded that even though the conductance of leachates from wheat caryopses was higher from those with low viability than from those with high viability, differences were not sufficient to be useful in evaluation of small differences in viability.

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