

# On the Mechanism of Aging in Soybean Seeds<sup>1</sup>

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## ABSTRACT

Changes in seeds of soybeans (*Glycine max* [L.] Merr. var. Wayne) which occur during accelerated aging (41 C, 100% relative humidity) showed subsequent loss of vigor, a decline in early respiratory activity, increased leakage of electrolytes, losses of as much as 10% dry weight from imbibing cotyledons, and a decrease in the swelling response of the imbibing system (seed plus H<sub>2</sub>O). Each of these changes with aging is interpreted as resulting from deteriorative changes in membranes.

The performance capability of seeds in general and soybeans in particular declines during storage. This aging or loss of vigor is evidenced by delayed germination and emergence, slower growth, increased susceptibility to environmental stresses, and, ultimately, a decline in germinability (8, 13, 18, 29). Seed aging is therefore a serious problem in agriculture, one that is receiving increasing research interest (13, 16); yet little is known about the actual mechanism or events leading to loss of viability and vigor. A number of different events or processes have been suggested as causal mechanisms, including chromosomal damage (21), loss of various enzymes (29), degradation of the respiratory system (2), loss of ATP production/storage capacity (6, 9), and deterioration of membranes (4, 7, 23, 28).

The rate at which seeds lose vigor during storage is affected by environmental factors such as temperature, moisture, and O<sub>2</sub>/CO<sub>2</sub> concentrations (13, 16, 28). Harrington (16) has suggested that within the normal range of moistures and temperatures for stored seed, each 1% reduction in seed moisture or each 5 C reduction in temperature doubles the storage life of the seed. Using such "rules-of-thumb" and assuming that the effects are additive, one can estimate that seed vigor would deteriorate about 500 times more rapidly at 40 C and 18% moisture content than it would at 20 C and 8% moisture. Thus, high temperature, high humidity storage provides a convenient method for accelerated aging of seed (8, 18, 29).

Those age-correlated physiological changes which appear earliest would seem most likely to have a causative relation to aging. We have previously developed methods of examining changes in some dynamic properties of soybeans during earliest stages of germination (20), and in the present study, these methods are applied to the problem of the mechanism of seed aging.

## MATERIALS AND METHODS

Certified seeds of Wayne soybeans (*Glycine max* [L.] Merr. var. Wayne) were obtained from Nebraska Seed Foundation and stored at room temperature and air dryness (6-8% moisture).

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There was no detectable deterioration in seed vigor during the few months of experimentation.

Accelerated aging was obtained by a modification of the method of Byrd and Delouche (8) where seeds were placed in a single layer on plastic Petri dishes and placed in a covered water bath maintained at 41 C and nearly 100% relative humidity. Water was taken up hygroscopically from the humid atmosphere within the water bath, but no free water came in contact with the seeds. After accelerated aging for various times, the seeds were removed and allowed to return to air dryness (6-8% moisture) at room temperature before further experimentation. Moisture content was determined by drying at 100 C for 24 hr.

For measurements of vigor, 50 seeds were placed in rolled paper toweling, wetted with distilled H<sub>2</sub>O, and held in the dark for 4 days at 25 ± 1 C with occasional additions of distilled H<sub>2</sub>O. The number of seeds germinated (embryonic axis longer than 1 cm), average root length, and axis weight (measured by removing cotyledons and weighing the remaining root and hypocotyl) were recorded.

Respiration was determined manometrically by adding 1 ml of H<sub>2</sub>O to six cotyledons (0.5 g) (20). Leakage of electrolytes from imbibing cotyledons was determined by placing six cotyledons in 10 ml of distilled H<sub>2</sub>O and measuring the changes in electrical conductivity of the H<sub>2</sub>O with a conductivity bridge (20, 24).

Changes in the total volume of an imbibing system (seed plus H<sub>2</sub>O) were measured using a dilatometer (20): 100 g of dry seeds were placed in a 1-liter flask; H<sub>2</sub>O was added to completely fill the flask, which was then stoppered. A burette was inserted through the stopper so that the H<sub>2</sub>O rose into the graduated portion of the burette. Changes in the total volume of the imbibing system could then be observed as changes in the H<sub>2</sub>O level in the burette.

## RESULTS

Three seed performance parameters, per cent germination, root length, and axis weight, were used to measure changing seed vigor with accelerated aging (Table I). Germination began to decline after 4 days of accelerated aging, but axis growth in terms of both weight and root length showed adverse effects after 3 days of treatment. The seeds of this seed lot were completely nongerminable after 7 days of accelerated aging.

Changes in respiratory activity following accelerated aging are shown in Figure 1. Manometric determinations of early respiration are complicated by nonrespiratory exchange of gases (desorption) and vapor equilibration during the first several min after the introduction of H<sub>2</sub>O (20); for this reason, comparisons of respiratory activity were begun after 45 min of imbibition. The data in Figure 1 indicate that there was a decline in O<sub>2</sub> consumption with increasing time of aging; after 7 days of accelerated aging, when germinability had been lost, the respiratory rate was less than half that of the controls. A comparison of Q<sub>O<sub>2</sub></sub> at various times after wetting (Table II) shows that the lessened respiration becomes increasingly exaggerated during the first 5 hr of imbibition.

Loss of electrolytes into the imbibing medium increased with duration of accelerated aging (Fig. 2). The period of rapid leakage of electrolytes in the first few min of imbibition, before the rate

Table I. Effect of accelerated aging (41 C, 100% relative humidity) on germination and growth (after 4 days) of soybeans.

Days Aged	Percent Germination	Average Axis Weight (mg)	Average Root Length (mm)
0	100	111	31.8±4.1
1	99	112	27.9±3.7
2	99	101	28.1±3.1
3	96	65	18.3±2.6
4	81	59	13.9±2.6
7	0	--	--

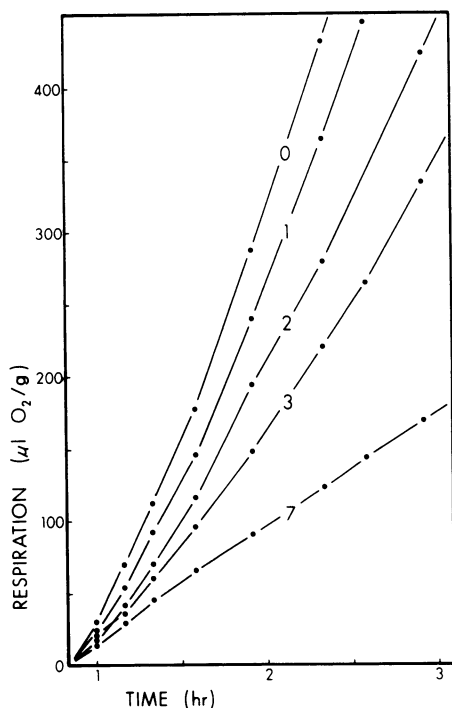


FIG. 1. Effect of accelerated aging (41 C, 100% relative humidity) on early respiration of soybean cotyledons. The seeds had been aged 0, 1, 2, 3, or 7 days as indicated.

Table II. Effect of accelerated aging (41 C, 100% relative humidity) on respiration of soybean cotyledons after various periods of imbibition.

Days Aged	Respiration rate ( $\mu\text{l O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ )				
	Imbibition Time (hr)				
	1	2	3	4	5
0	230	355	400	440	480
1	190	300	350	390	420
2	140	230	260	310	330
3	110	180	215	245	265
4	100	160	170	190	205
7	90	80	90	100	120

becomes linear, is considered to be the period during which the membrane systems are becoming reorganized upon hydration (20, 24). This initial leakage is increased by each increment of the accelerated aging treatments. The cotyledons from seeds which had experienced 7 days of accelerated aging continued to show a rapid, nonlinear leakage for the entire measurement period.

Relative water uptake in a 4-hr period as a function of aging is shown in Table III. If one expresses imbibitional weight gain as a per cent of the initial fresh weight, it appears that aged cotyledons take up less  $\text{H}_2\text{O}$  than non-aged. If  $\text{H}_2\text{O}$  content is determined as

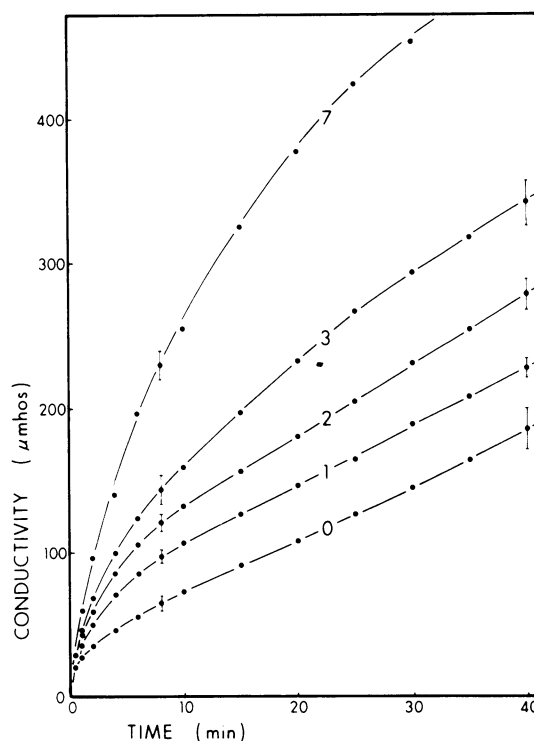


FIG. 2. Effect of accelerated aging (41 C, 100% relative humidity) on leakage of electrolytes from soybean cotyledons during early imbibition. The seeds had been aged 0, 1, 2, 3, or 7 days as indicated.

Table III. Effect of accelerated aging (41 C, 100% relative humidity) on weight gain and dry weight loss after 4 hr imbibition of soybean cotyledons. Each datum is mean of 2 replicates of 20 cotyledons each.

Days Aged	Moisture content expressed as:			
	% Fresh Weight		% Dry Weight	
	Initial	Final	Dry	$\text{H}_2\text{O}$
	Weight	Weight	Loss	Imbided <sup>1</sup>
0	120.4	136.9	1.4	1.35
1	117.3	138.8	3.7	1.34
2	114.4	145.6	7.9	1.34
3	112.6	150.1	10.4	1.34
4	108.1	150.5	12.3	1.33

<sup>1</sup> g  $\text{H}_2\text{O}$  imbided per g initial dry weight (determined from increase in fresh weight corrected for dry weight loss)

a per cent of the final dry weight, the opposite appears to be true. The discrepancy between the two types of  $\text{H}_2\text{O}$  uptake data is due to the loss of substantial amounts of matter from the cotyledons during imbibition. By estimating the original dry weight of the cotyledons (from known moisture contents of identically pre-treated seeds), the loss of dry weight during 4 hr of imbibition could be calculated. Such loss was found to increase about 10-fold in the seeds which had experienced 4 days of accelerated aging. The amount of  $\text{H}_2\text{O}$  taken up per seed is independent of aging when imbibitional weight gain is corrected for dry weight loss as shown in the last column of Table III.

As we have previously reported (20), the total volume of an imbibing system (seed plus water) follows a highly repeatable pattern, the most prominent feature being a marked increase in volume during the first 4 hr, which was interpreted as a ballooning of the seed caused by gases trapped during inward movement of the  $\text{H}_2\text{O}$  front. Aging resulted in a marked lessening of the volume

increase (Fig. 3). After 7 days of aging, no volume increase was observed at all.

## DISCUSSION

The evidence provided here indicates that accelerated aging treatments (which progressively lower seed vigor) cause a marked lowering of early respiration of isolated cotyledons, large increases in the initial leakage of electrolytes, increases in dry weight loss, and depression of the systemic volume increase associated with imbibition. Each of these age-induced changes is consistent with the interpretation that there is a deterioration in the membrane systems of the seed with aging, and the suggestion is offered that a basic mechanism of aging in soybean seeds is a deterioration of the membrane's ability to reform and function upon hydration. The parallel declines in seed performance and in membrane performance parameters suggest a causal relationship. The appearance of the effects early in the germination sequence, *i.e.* immediately upon imbibition, also lends evidence to the concept that membrane deterioration is primary or causal rather than a secondary effect.

The increased leakage associated with aging might be the result of a more permeable membrane or of a larger pool of electrolytes. Ching and Schoolcraft (10) considered this possibility in amino acid leakage studies of vigorous and aged, nongerminable seed. They found that while the pool of free amino acids did approximately double with aging, the loss of amino acids into the imbibing medium increased 160-fold; at the same time, total electrolyte leakage increased about 7-fold. In their studies it is apparent that amino acids represented only a small portion of total electrolytes leaking out and that leakage increased much more than did the pool of potentially leachable materials. Ching and Schoolcraft (10) concluded, as have several others (3, 17, 30), that leakage is an index of seed vigor. Abdul-Baki and Anderson (1) cautioned that leaching of sugars from barley did not correlate well with vigor, but that sugar utilization and mechanical injury appeared to affect leakage more than age of seed. With that possible exception, aging does appear to result in leakage which parallels vigor losses.

Most workers who have performed leakage studies examined the loss of materials from previously imbibed seeds or after extended periods of imbibition. In such cases, leakage might be due to loss of metabolic energy for membrane transport mechanisms and maintenance of cellular integrity or the result of auto-

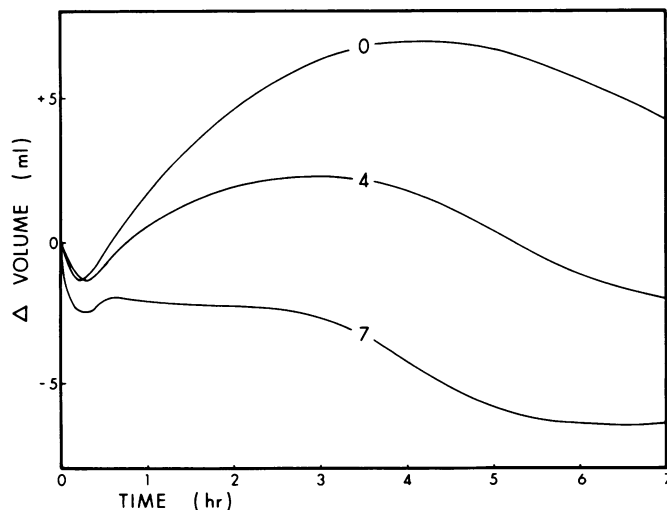


FIG. 3. Systemic (seed plus H<sub>2</sub>O) volume changes associated with imbibition of soybeans after 0, 4, or 7 days of accelerated aging (41°C, 100% relative humidity).

lytic damage to membranes after imbibition is complete. If membrane damage is a primary effect of aging and a primary cause of vigor loss, it should be expressed immediately upon hydration. The present data show that leakage of electrolytes is increased by aging during the initial burst of leakage before membranes reorganize to their hydrated orientation (20, 24) as well as during the subsequent period of linear H<sub>2</sub>O uptake and leakage.

Besides their role in limiting the loss of intracellular components, membranes also bound the intracellular compartments and organelles. Any delay or obstruction of membrane reorganization during imbibition would allow mixing of substances normally segregated from one another by membranes in the hydrated cell. The direct result could be loss of metabolites, inability to maintain electrical, chemical, or pH gradients, and a mixing of normally separated cellular constituents. An indirect result would be loss of vigor.

At the ultrastructural level, Harman and Granett (15) have reported that aged pea seeds show evidence of damage to mitochondria and the plasmalemma. Abdul-Baki and Baker (4) found that mitochondria of aged seeds are more fragile, *i.e.* less amenable to preparation for electron microscopy. Others have found mitochondrial membranes to be somewhat anomalous or less well organized in old seeds (5, 14). The changes in membrane and organelle organization are not very striking, however, until after hydration has taken place (14, 22). Simola (22) found that postimbibitional development or reorganization of mitochondria and plastids was inhibited in old pine seed. Hallam *et al.* (14) reported that an autolytic process seemed to occur after imbibition of nonviable rye embryos; organelles ruptured and the contents leaked into the cytoplasm and then into the wall space. The possibility that aging is caused by deterioration of lysosomal membranes has been raised by other workers both for hydrated animal (11) and hydrated/dry seed tissues (28).

A decline in O<sub>2</sub> consumption with aging has been reported by a number of workers (8, 27, 29). Respiration within the mitochondrion is a function of "unit membranes" and loss of membrane integrity would presumably alter the functional relationships of the membrane-bound components of the respiratory chain. Some workers have suggested that mitochondria of older seeds are progressively uncoupled (5) which could easily be reconciled with loss of membrane integrity (especially if the transmembrane proton gradient or electron motive force is crucial for coupling).

We have found in separate studies that cell turgor, an obvious function of membrane integrity, declines in parallel with seed vigor. The ability of the plasmalemma to retain osmotically active materials and/or its ability to retain water against an applied mechanical force was adversely affected by even one day of accelerated aging.

The increase in systemic (seed plus water) volume during imbibition correlates directly with seed vigor and viability. We have proposed that the dilation or ballooning of seeds which must cause the system's volume to increase is the result of pressurized gases being trapped in the interior of the seeds during hydration (20). The ability of seeds to develop internal pressures may be reduced in aged seed as a consequence of the deterioration of membrane effectiveness and lowered capability for maintaining turgor.

Several workers have proposed that the lipids within the membranes are likely sites of aging damage and/or that autoxidative/peroxidative reactions are the likely chemical mechanism (2, 16, 18, 23, 28). Oxidation of fatty acids in membranes, especially of lysosomes, had been proposed as a mechanism for aging in animal systems (11, 25, 26). Besides directly destroying lipids and altering their ability to function in the membrane, the autoxidation of fatty acids produces highly reactive free radical intermediates (12, 25).

There is little direct evidence at present for an autoxidative, free radical mechanism for aging. Pammenter *et al.* (19) were able to reverse vigor losses by placing dry, aged seeds on a cathode

charged to 300 v. They suggested that the effect was to reduce free radicals in the seeds. If such be true, much of the age-induced damage in the seed must be latent, not actually occurring till hydration. The absence of free H<sub>2</sub>O would restrict the free radical chain reactions in a dehydrated, crystalline lipid arrangement (23). As free H<sub>2</sub>O is restored during hydration (or in high humidity storage where H<sub>2</sub>O may be taken up hygroscopically), the rate of damaging processes may be expected to accelerate.

The present experiments are consistent with the concept that seed aging may be associated with deteriorative changes in membranes.

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