Symposium: Deterioration Mechanisms in Seeds

Deterioration of Seeds During Aging

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The seed represents a remarkable stage in the life history of a higher plant. This stage of plant development serves primarily as a means for reproduction of the species. Some species adapted to environments that allowed the seed to desiccate and survive adverse environmental conditions in which the species normally could not grow. Once environmental conditions improved for plant growth some seeds would germinate, differentiate, flower, and set seed, which starts the process over again.

When man learned to cultivate plants he also learned that he had to save part of the crop to sow in the following year. Modern man has bred plants for increased yield, easier harvesting, minimal dormancy, softer seed coat, and better nutrition, but has left many species vulnerable to seed deterioration (loss of seed vigor and eventually loss of germinability) prior to harvest as well as during storage.

During the past decade much research has been devoted to

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studying seed germination, dormancy, storage, and metabolism. It is interesting that much of the published work is not by agronomists or horticulturists who generally have been interested in seed biology. Instead, the work is being done by plant physiologists, biochemists, and cytologists whose interests lie in the more "basic" or "fundamental" areas of research.

In many of the studies described in this review, a technique called rapid (or accelerated) aging has been employed, which involves subjecting seeds to conditions of high moisture and temperature. These conditions are highly conducive to the growth of microorganisms on and in seeds, which is not appreciated by many researchers outside the field of plant pathology.

Effects of Storage Environment on Seed Longevity

It is well known that most seeds used in agriculture today can be stored many years if the seeds are kept at moisture contents of 5–8% (36). Seed involved in interstate and foreign commerce must be tested every 5 mo according to federal law. However, seeds maintained in hermetically sealed containers at 5–8% moisture, depending on the species, and packed within 9 mo after harvest, do not have to be retested for germination until 24 mo has elapsed. We point this out to demonstrate the fact that properly stored seeds can be maintained in good condition for several years. Seeds can be maintained for longer periods at subfreezing temperatures (36).

Moisture content of seeds is dependent on the relative humidity (RH) of the air in which they are stored. The higher the RH the higher the seed moisture and the faster the rate of deterioration, particularly at moisture contents above 12%. Storage fungi can be a problem in stored seeds with too high a moisture content. For example, soybeans stored at 12.5% moisture or higher, and wheat and corn at 13.5% moisture or higher, can be infected by storage fungi (16).

Physiology of Deterioration

The complete loss in ability to germinate is the ultimate result of seed deterioration. However, before that state is reached, the individual seeds lose vigor at different rates. This can show up as increased sensitivity to adverse storage conditions, a slower and uneven rate of germination, poor seedling emergence, slower seedling growth, and an increase in the number of abnormal seedlings (particularly under adverse field conditions).

Seed deterioration can occur in the field after they reach physiological maturity, particularly if harvest is delayed by wet weather. This is commonly referred to as weathering. The effects of weathering can be just as bad for seed quality as poor storage conditions.

There is increasing evidence that many seed problems can be minimized or overcome at the time seeds are sown by increasing the moisture content to percentages greater than those required for good storage. Pollock (27) showed that chilling injury in lima bean could be prevented by equilibrating embryonic axes to 20% moisture by allowing the axes to absorb water from a high-RH atmosphere. Anderson (5,6) studied adenylate metabolism in embyonic axes of soybean. Although not stated in the methods, these studies were done with embryonic axes that were placed at high RH in a cold room at least the night before experiments were performed. These high-moisture axes were quite flexible, not hard and brittle like those from a dry seed. Pretreatment at high RH was done because the results from experiments were more uniform and higher ATP levels were attained than when very dry axes were used. Different experimental approaches have shown that short periods of imbibition in water, or equilibration in a high RH atmosphere, followed by dehydration before germination also benefits germination (10). Also, rapid-aging effects could be partially overcome by allowing imbibition to proceed slowly in 30% polyethylene glycol (39). Such studies indicate that much of the damage caused by deterioration might become manifested during initial imbibition, at least when very dry embryos, axes, or seeds are used. This equilibration of seeds at a high RH might enable cellular components, particularly membranes, to withstand the forces of rapid water uptake during imbibition, which normally might cause subcellular damage to partially deteriorated seeds.

For the past 40 yr or so efforts have been made to lengthen the storage life of seeds held under high-moisture conditions. Hydration-dehydration treatments that help prevent or delay deterioration under poor storage conditions have been reported (10). Also, storing seeds at a negative electric potential (cathodic protection) has been reported to be beneficial (26), but we have been unable to repeat this work.

Little research has been done on the effects of plant hormones or growth regulators on germination of deteriorated seed. Germinating but aged rape seed produced less C_2H_4 than did fresh seed (35). Application of C_2H_4 to aged seed increased the rate of germination, but total germination was not affected (35). Gibberellic acid (GA_3) solution did not increase germinability of deteriorated wheat seeds (4), although it did stimulate growth of seedlings from such seeds, but to a lesser degree than in seedlings from nondeteriorated control seeds. It seems that C_2H_4 or GA_3 application will not reverse deterioration, at least under the conditions studied.

Biochemistry of Deterioration

An area of seed deterioration research that recently has received much attention is the measurement of metabolic activity during imbibition. Studies range in complexity from measuring respiration rates to determining in vitro translation products. It is apparent from studies with seeds that have undergone some deterioration that the in vivo metabolic activity of such seeds is not as great as in seeds that have not undergone deterioration. If deterioration has not progressed too far, cells apparently can repair some damage and produce a seedling (11).

Rates of O_2 uptake have been reported (38) to be good quantitative indices of vigor. This often seems to be the case, particularly when seed lots begin to decline in germinability, but in some cases these rates do not decline (1). Interestingly, the respiratory quotient (RQ) seems to increase or is maintained with deterioration. This occurs either because O_2 uptake declines faster than CO_2 production (3,38), or because CO_2 production increases while O_2 uptake either remains unchanged or decreases slightly (4).

Probably the largest change reported to occur with aging is the drastic reduction in glucose utilization of intact barley and wheat seed (1). This change occurs long before changes in germinability are observed. Most of the change in intact wheat and barley seed, however, is localized in the endosperm and probably has little to do with deterioration of the embryo (7). Because of masking of embryo metabolism by endosperm, metabolic activity of the intact seed with a large endosperm is not a good measurement of the metabolic activity of the embryo. Similarly, the presence of cotyledons can mask metabolic activity of the embryonic axis in seeds like soybean (3). More useful information can be obtained from studies with isolated embryonic axes. However, changes in the endosperm can affect the behavior of the embryo (17) and endosperm effects should not be completely ignored in deterioration studies.

It is clear from studies with isolated tissues that most biological polymer synthesis is adversely affected by deterioration. French (18) apparently was the first to report such effects. He studied starch synthesis by barley embryos from heat-damaged seed. Early work on isolated systems showed that deterioration lowers the rates of polysaccharide, protein, RNA, and lipid synthesis during the first hours of imbibition. The causes of the lower rates of polymer biosynthesis in isolated systems are not fully understood. However, all of these biosynthetic systems require ATP. Dry embryos and axes contain very low levels of ATP, but upon imbibition ATP content increases dramatically, from a level that is difficult to detect, to nanomole amounts (5,15,37). During imbibition, the ATP pool sizes seem to be lower in deteriorated than in nondeteriorated seeds (5,15,37). The causes for the lower ATP pools in deteriorated seeds are not fully understood. Most of the ATP synthesized during imbibition probably is of mitochondrial origin because ATP levels and turnover are decreased with cyanide

and anaerobiosis (23, and J. D. Anderson, *unpublished*).

The low rate of in vivo protein synthesis in aged seeds (24) is not fully understood, but is probably related to low ATP and GTP levels and/or otherwise damaged biosynthetic systems. This is somewhat controversial since soluble enzyme systems as well as ribosomes involved in protein synthesis (as measured by polyphenylalanine synthesis in a polyuridylic acid [poly U]-directed system) appeared to be fairly stable in deteriorated but nonimbibed soybean (5) and pea (12) embryonic axes, whereas profound differences were found in rye embryos (31). However, poly U-directed systems may not necessarily provide a true measure of protein synthesis. For example, poly-U might be able to operate with imperfect ribosomes but a native mRNA might not. Also, in the poly-U system high Mg^{++} concentrations might replace the requirement for elongation factor-1 (EF-1) in seed lots that have lost their ability to germinate (13). The role that EF-1 plays in the loss of in vivo protein synthesis and germinability is unclear because a 10% difference in EF-1 activity from rye embryos differing in germinability by 30% would have to account for a 77% loss in amino acid incorporation into protein (30).

The effects of deterioration caused by rapid aging on protein synthesis during the early hours of germination were studied by using high-resolution, two-dimensional electrophoresis (25, and J. D. Anderson, *unpublished*). Differences observed appeared to be largely quantitative, with less total protein synthesized in deteriorated than in control embryos. There were no obvious differences in types of proteins synthesized. Although the two seed samples had the same percent germination, those in the aged sample exhibited less vigor than the control.

There have been many studies of various enzyme activities in deteriorated seed. Abdul-Baki and Anderson (2) listed some of the constitutive enzyme activities reported to decrease with deterioration. However, the activities of many constitutive enzymes either do not decrease or decrease only slightly during deterioration; these enzymes probably have little to do with loss of seedling vigor and viability. Other enzymes such as adenosine kinase, adenine phosphoribosyltransferase (5), and superoxide dismutase (SOD) (J. E. Baker, *unpublished*) extracted from rapid-aged soybean axes and assayed by previously published methods (9), fall into this category. Stewart and Bewley (34), however, could not detect SOD activity in dry soybean axes. Other discrepancies exist; for example, glutamic acid decarboyxlase activity is reported to decrease with loss of viability in corn (38), but appears to increase in activity with deterioration of soybean embryonic axes (3).

The involvement of nucleic acids in deterioration has been studied, particularly in the rye embryo. There appears to be marked degradation of rRNA of nonimbibed embryos of deteriorated seed (31). However, rRNA profiles from rye embryos with 53% germinability were almost indistinguishable from profiles of embryos with 100% germinability (32). Possibly, the conditions under which the seeds deteriorated in the latter case were different than in the earlier studies. Some of the seeds used in the former case had moisture contents that could have supported growth of storage fungi. Bray and Chow (13) present evidence of a breakdown of rRNA in pea seed that were unable to germinate, but they did not precisely state the conditions under which the seed lost viability. Data on DNA degradation are unavailable for seeds having low rates of protein synthesis as a result of deterioration during storage but still had high or intermediate germination. In seeds that had lost all germinability, total DNA did not differ much from normal seeds, but spoolable DNA was reduced drastically (30). Electrophoresis of DNA from similar deteriorated seeds revealed some DNA degradation (14). We feel that no conclusions can or should be drawn concerning interrelationships between integrity of DNA, activities of ribosomes and elongation factors, and the loss of viability or induction of mutations when only viable and non-viable seeds are used. It is especially important to interpret data cautiously when conditions of deterioration or the lengths of time the seeds were nonviable are not known.

Although seed lipid constituents are probably studied the most, changes that take place during the course of deterioration remain among the most controversial aspects of deterioration. Changes in the concentrations of unsaturated fatty acids, particularly in phospholipids, is a case in point; such changes are thought to be caused by free radical attack. Harman and Mattick (22) using total lipids from peas, and Stewart and Bewley (34) using phospholipids from soybeans, reported losses in unsaturated fatty acids, particularly linolenic acid. In contrast, Priestly and Leopold (28) using both total lipids and phospholipids from soybeans reported no change with deterioration. It should be pointed out that in Stewart and Bewley's (34) study, seeds stored at high temperature but low RH did not decrease in germinability, but the linolenic acid concentration decreased to about the same level as that observed after 1 day at high RH and temperature. These latter conditions reduced germinability by over 50%. With this in mind, the significance of such a drop in linolenic acid in the deteriorated sample is questionable as far as a cause of deterioration, and might be associated with high temperature instead of deterioration.

Seeds are known to contain various antioxidants that would quench free-radical attack. Possibly a loss in such components would lead to death of seeds. Priestly et al (29) measured antioxidant levels and found no change in soybean samples that had completely lost the ability to germinate. Malondialdehyde (a product of lipid peroxidation) levels in dry axes are about the same even after germinability is completely lost (34). Priestly et al (29) did not detect differences in organic free radicals in any of their seed samples and contended that free radicals probably were not involved in deterioration. Thus, the role of free radicals in seed deterioration particularly in causing lipid peroxidation has not been proved and is not supported by convincing evidence. Clearly, more research is needed to clarify this problem.

Ultrastructural Differences

During the past 25 yr there has been great interest in studying the structure of seeds by electron microscopy. In 1970, we published a paper showing some of the differences in ultrastructure of wheat embryos that deteriorated in the presence of storage fungi (8). We showed that the plasma membrane of many cells from deteriorated seeds had withdrawn from the cell wall and was ruptured. This withdrawal of the plasmalemma was also observed occasionally in cells from control embryos suggesting that some cells of the control seeds were injured or dead. The grainy material found between the plasmalemma and the cell wall might be material that had leached from the cell. This was subsequently reported by other investigators (19,21).

One other very obvious change is the coalescence of spherosomes or lipid bodies (8). Others also have observed this, but it occurred in seeds that were not infected (21) or was observed only during imbibition (19,33). On the basis of these observations, the causal relationship between ultrastructural change and storage fungi is uncertain. Possibly, storage fungi accelerated the normal ultrastructural changes that would have occurred in their absence. However, we found evidence of coalescence only in embryos that were infected and possibly more deteriorated; seeds that were lightly infected (ie, those showing a few conidia growing from the embryo end) or less deteriorated had fewer coalesced spherosomes than the more heavily infected ones.

Other reported ultrastructural changes involve ribosomes and the membranes of the nucleus, mitochondria, and other organelles (19,21). In our work we saw little evidence of such changes. Some of the changes observed might be due to different fixation techniques, different species, and (of course) different storage conditions.

Little is known of the interactions between microorganisms and ultrastructural changes that occur during deterioration. As far as we are aware, the only ultrastructural studies of deteriorated or nonviable seed that included tests for storage fungi were those of Anderson et al (8) and Harman and Granett (21). Others either were not aware of the possible role of such microorganisms, or stated that they never found evidence of fungi in electron micrographs, even though they used seed that deteriorated under conditions conducive to growth of storage fungi. For the most part, storage fungi are found under the seed coat or associated with the

pericarp. As far as we know, no one has presented anatomical evidence of the localization of storage fungi within embryos (20). We wish to present such evidence from wheat seeds infected with storage fungi (Fig. 1). The hyphae were found only in the intercellular spaces. This internal infection was not of wide occurrence and occurred late in the infection process. The late occurrence of this phenomenon suggests that it had little to do with loss of seed germinability. Additional studies are needed to clarify some of the doubt that exists as to the relationships among the presence of storage fungi and ultrastructural and biochemical changes associated with deteriorated seed.

Genetics of Seed Longevity

Much has been written about mutations that can be induced in seeds during deterioration. While we must be concerned about such mutations especially in our germ plasm storage facilities, the plant breeder also can go the other way, ie, breed for good seed storability. Plant breeders have known for about 50 yr that some inbred lines of corn store better than others. It is now a foregone conclusion that seed storability is a genetically transmissible trait that can and should be exploited, especially in developing countries in the tropics. Currently, scientists at the International Institute of Tropical Agriculture in Ibadan, Nigeria, are breeding for weathering resistance and good storage potential in soybeans (E. Kueneman, *personal communication*).

The mechanisms by which viability is maintained are not known. Hardseededness is known to improve storability and is probably the reason some seeds have survived for centuries. However, this is not the complete story. Other factors are involved. The structures in which seed develop (eg, pods, glumes, etc.) probably play an important protection role in weathering resistance. Possibly, lines that weather well have more protection. Protective structures would be of minimal importance during storage, but they may provide higher quality seed going into storage. It is known that high-vigor seed store better than low-quality seed. The role genetics play in seed quality is a relatively unexploited area, and those of us working in seed quality should take more advantage of genetic variation in storability.

Conclusions

The mechanisms by which seeds lose viability are not known. We do know that high moisture and high temperatures during storage are very detrimental to the survival of most seeds. It is also more or less agreed that as seeds deteriorate they become "leaky" during the first hours of germination and seem to lose their abilities to carry on in vivo biopolymer (polysaccharide, protein, nucleic acid, and lipid) and ATP biosynthesis. Difficulties arise in trying to determine the cause for the low in vivo metabolic activity of deteriorated seeds. During the past decade, several groups have studied this low activity using some of the most modern and sophisticated biochemical techniques available. Even with this new technology there are still questions among the various groups as to what occurs with loss of seed vigor and germinability. Various groups present different answers, all of which are probably correct for the particular seed lots used. The differences in results are attributable in part to differences in the conditions under which the

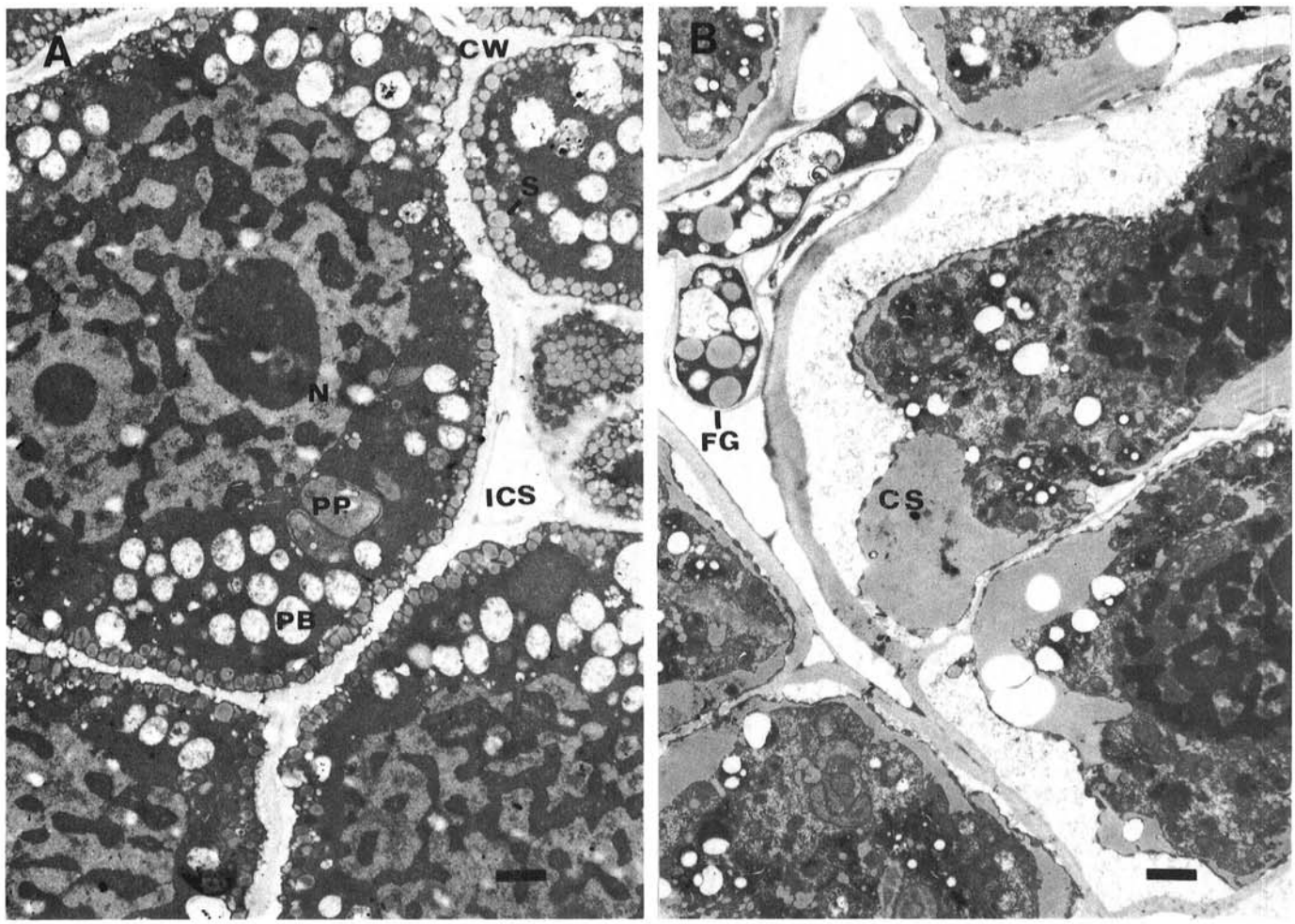

Fig. 1. Electron micrographs of wheat embryo tissue of **A,** control and **B,** storage fungi-infected seed showing cell wall (CW), intercellular space (ICS), proplastids (PP), protein bodies (PB), spherosomes (S), coalesced spherosomes (CS), and fungal hyphae (FG). Fixation and staining procedures were previously published (8). Bars represent 1 μm.

seeds were produced and the conditions under which they deteriorated, especially if seeds were stored under conditions conducive to the growth of storage fungi. Also, confusion arises when conclusions are made from studies in which only viable and nonviable seed lots were compared. This confusion is compounded because most of the conditions under which the seeds deteriorated were not given or known, and the length of time the seeds had been in the nonviable state was not given or known. Thus, one cannot know when the changes that were measured took place or which of them were involved in the loss of viability or integrity of genetic material.

For the future, studies are needed to determine the cause of the reductions in rates of biopolymer (eg, protein, RNA, and polysaccaride) synthesis that occur before large declines in germinability. There are few data available that explain these reductions and such studies should yield information that will be useful for proposing mechanisms of deterioration.

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