4-1-1987

The Mortal Seed

J. C. Delouche

THE MORTAL SEED

James C. Delouche[1]

Seeds die - as every farmer, gardener, and seedsman knows. Some succumb to the moisture, heat and molds before harvest; some are mortally injured during handling and threshing; some expire in the seedbed in the process of germination; and some just fade away with aging during storage. While the dying of seeds is a matter of great concern to the farmer or seedsman, the point of interest to most laymen and the media is not that seeds die, but that they live so long, or put another way, their seeming immortality rather than their mortality - which is, of course, not at all surprising.

Seed Longevity Records

A few months ago the Dean sent over the seeds collection of an alumnus (and benefactor) made when he was a student more than 60 years ago. The alumnus wanted to know if the seeds were still germinable. The seeds of White Kafir and Brown Dura sorghum, sanfoin, serradella, Prussian Blue field pea, Ito San soybeans and Whippoorwill cowpeas were all dead, but about 5% of the unhulled white biennial sweet clover seed produced very weak seedlings. The alumnus was very excited about the sweet clover and asked that we return the remaining seeds to him - for what reason we do not know.

This was not the first occasion that we got involved in determining the viability of old seeds. Indeed for many years now our laboratory has been a frequent recipient of vials and envelopes of seeds found while cleaning out trunks, files, even safety deposit boxes. Most of the seeds were over 50 years old and in about 99% of the samples the seeds were "stone" dead. As might be expected, the few samples with germinable seeds were species with "hard seeds" such as sweet clover, dock, and bindweed, but we have not been so lucky as others to turn up good data on well documented seed samples suitable for publication. A surprising number of professional papers have been published on the results of germination tests made on old seeds that more-or-less just happened to turn up in cleaning out files and storerooms in Departments of Botany, Agronomy and Horticulture, or

----

[1]Professor and Agronomist, Seed Technology Laboratory, Miss. State University, Miss. State, MS.

research labs. Although fortuitous, these papers are always well read, and have added greatly to our knowledge of the longevity of seeds.

The media have long been attracted to feats of longevity including tales of long-lived seeds. In her recent, well documented and immensely fascinating paper "Ancient Seeds; Seed Longevity" (JOST 10:1986), Vivian Toole cites an article in an 1843 issue of the Gardner's Chronicle that reports on the germination of wheat and barley seeds found in an ancient tomb in Egypt which opened up the interesting possibility of making bread from the produce of seeds (grains) that might have been stored in Joseph's granaries during the fat years. Mrs. Toole cites many other equally sensational stories about "mummy" wheat and other seeds of antiquity, and then "examined the facts" which are that none of the reports of germination of seeds from King Tut's tomb and other tombs of similar antiquity are valid. Generally, the antique seeds are completely "carbonized" and very fragile. Most reports of their germination were the results of errors in authenication and dating or over-heated speculation, but in a few instances deliberate deception appears to have been involved a la the Piltdown man hoax. While seeds may not survive into the antiquity stage, they are very long lived compared to other living things.

The oldest longevity claimed is 10,000 years for seeds of an artic lupine found in a lemming burrow in the Yukon. The dating of the seeds, however, has not been rigorously validated. The most widely publicized - at least among seed technologists and scientists - seed longevity claim is for seeds of Indian lotus found in an lake bed deposit in Northeast China by I. Ohga, a Japanese botanist, in 1923. The generally accepted age of the seeds is 1000± 200 years. Seedlings and plants from the 1000 year old lotus seeds are shown in Plate 1 of Lela Barton's classic "Seed Preservation and Longevity" (1961). The Indian lotus seed claim is my favorite and probably will remain so even if it is supplanted by one much older. While the geography might not be too correct, I like to imagine that some of the lotus seeds might have been trampled in the lake bed by the mount of one of Genghis Khan's golden horde on their way to the Danube about 800 years ago.

There are a substantial number of well documented seed longevities in the 100-200 year range and many in the 50 to 100 year range. The main sources of the seeds were collected specimens in hebaria and/or museums for which collection dates could be established. In one interesting case cited by Eric Roos in his recent paper "Precepts of Successful Seed Storage" (CSSA Sp. Pub. No. 11): 123 year-old seeds of oats, barley and wheat sealed in a glass tube in the foundation stone of the Nuremburg City Theater germinated 21, 12 and 0%, respectively.

Seed kinds that have been shown to be long-lived in air-dry storage (e.g., hebarium case) are usually from species that are members of families in which the main dormancy mechanism is water impermeability of the seed coat (hard seeds), viz., legume, morning-glory, and mallow (cotton) families. The good longevity of hard seeds can probably be attributed to the maintenance of a uniformly low and stable seed moisture content as contrasted to species with "soft seeds" which increase and decrease in moisture content in accord with the seasonal changes in climate. Most of the germinable seeds we have found in the old seed collections sent to our lab - as mentioned above - are of species such as sweet clover, other clovers, bindweed, alfalfa and some of the weedy malvas. The grass seeds in the collections are always dead.

In a modern air-conditioned office laboratory, seeds remain germinable for a surprisingly long time - even seed kinds considered to be short-lived as soybeans and peanuts. Good quality soybean seeds maintain germination at an acceptable level for 4-6 years and a few survive up to 7-8 years.

The longevity of seeds in soil is of great interest to ecologists and weed scientists and has been extensively and intensively studied. Germination test results of seeds from buried seed experiments represent some of the best documented instances of seed survival for long periods.

Buried Seed Studies

The natural manifestation of the longevity of seeds is not in herbarium cases, trunks in attics, foundation stones of city halls, or Egyptian tombs, all of which involve storage of "air-dry" seeds in air - a human intervention in the ecology of plants. Rather, the main respository in nature for seeds from dispersal to germination is the soil - including lemming burrows and peat bogs in old lake beds.

Seed longevity on or in the soil is an important property in the evolution, survival, distribution and succession of plant species. And, it has important implications on both the negative and positive sides in the man-ordered ecology of agriculture.

On the positive side, the continuance of annual species in permanent meadows and pasture lands is dependent on the survival, i.e., longevity, of the seeds from dispersal until the start of the next annual cycle. Plant breeders have screened for variability in seed longevity in annual forage species and deliberately incorporated increased longevity in so-called re-seeding varieties. The assurance of an adequate population of the annual species through "natural" re-seeding eliminates the cost of seeds for annual or periodic seeding. Since the costs of seeds for some good forages are rather high, the development of re-seeding varieties has permitted their use on a wider scale than would be possible with annual replanting. The concept of re-seeding plant types has been extended from pastures to wildlife refuges and habitats. Some annual species that provide excellent cover and feed for wildlife could not be used except on very exclusive hunting preserves if annual seeding was necessary because of prohibitive costs of the seeds.

The negative side of seed longevity on or in soil is well known to every gardener, farmer and weed control specialist. Retention of the germinability of seeds in or on the soil for at least several years in an important and near invariable characteristic of weedy species. Since most weeds are also prolific seed producers, even a fair weed population in one season makes a hefty deposit in the seed "bank" in the soil, which then can be drawn upon to establish a weed population following a season of near complete weed control. The well stocked bank of seeds in agricultural soils and their excellent longevity insure continuance of the farmer's battle with weeds and employment for weed control specialists regardless of the structural changes in U.S. agriculture. It should be noted that good longevity of seeds of crop varieties in soil contributes to the volunteer problem with the volunteers acting as weeds.

Much of our knowledge of the longevity of seeds in soil has come from the results of buried seed experiments. In 1879, Professor W. J. Beal of Michigan Agricultural College (now Michigan State U.) placed seeds of 23 local weeds in slightly moist sand in bottles and then buried the bottles in an inclined, upside down position about 20 inches deep in a sandy knoll on the campus. Seeds were retrieved every 5 years until 1920 and then every 10 years with enough seeds (bottles) until about 2040. In 1980 - after 100 years - some seeds of three species were capable of germination: a mallow (Malva rotundifolia) and two mulleins (Verbascum spp.). Seeds of black mustard and one of the smartweeds exhibited some germination through 50 years, while a small percentage of the curly dock and evening primrose seeds maintained germinability through 70 years. The second classic buried seed experiment in the U.S. was started by J.W. T. Duvel of the USDA in 1902. Seeds of 107 crop and weed species were mixed with sterilized soil in porous clay pots, covered with saucers, and buried 8, 22 and 42 inches deep at the USDA, Arlington, VA research farm. Interestingly, the experiment had to be terminated in 1941 (39 years) to make way for the Pentagon. Some seeds of 51 of the 107 species were germinable after 20 years, while 36 species were still germinable at 39 years when the experiment was terminated. While many of the seeds of the 31 cultivated species in the experiment were dead after one year, a few species exhibited some germination after 20 years: alsike, red, white and bush clover; celery, Ky. bluegrass, timothy and tobacco.

Other buried seed experiments were set up by W. L. Goss in Shafter, CA in 1932, and by Egley and Chandler at Stoneville, Miss. in 1972. As in the case of the Beal and Duvel experiments, species exhibiting the greatest longevity have "hard" seeds or other profound types of dormancy.

The longevity record (circa 1000 years) for buried seeds is, of course, the Indian lotus seeds taken from a lakebed in China in 1923 by a Japanese botanist as described in the previous column.

The substantial longevity of seeds buried in soil seems rather strange at first thought to seedsmen and seed technologist who associate seed longevity with low moisture content and, secondarily, low temperature. The moisture content of seeds buried in soil must be quite high and remains so at least in the eastern half of the U.S. where rain is distributed throughout the year. As suggested in the opening section of this column, however, and argued persuasively by T. A. Villiers (e.g., Seed Ecology, PA State U. Press), the reverse seems strange to the seed ecologist, viz., that the life span of seeds is so long under the unnatural conditions of air storage. Villiers and associates and others have demonstrated the superior storability of fully imbibed seeds, i.e., maximum seed moisture, as compared to air dry seeds of some species. In all cases the seeds are held from germination by a primary or induced state of dormancy. The longer life span of at least some kinds of seeds in a fully hydrated as compared to air dry condition has important implications in terms of theories of seed aging.

The Dying Seed

Seed are mortal. Death appears inevitable. But, the terminal condition does not come about suddenly - except as a consequence of drastic mechanical or thermal abuse. Rather, there is a transition phase of some duration from life in full bloom to death - a stage which Bob Moore termed the dying seed. While the concept of the dying seed is not new, its significance in terms of the biological function of seed has been appreciated only relatively recently, and not fully even now. Dr. Moore's admonition to seed technologists not to misuse the terms "dead" and "live" in connection with seeds was set forth in a beautifully written paper delivered before the Association of Official Seed Analysts' meeting at Fort Collins, CO in 1963. In "The Dying Seed" he described the self-arguments and thinking that led to his many insights into the processes involved in the, "hidden transformation of initially sound seeds from a germinative to non-germinative condition." He related how, "in due time I was to learn that the division between germinative and non-germinative seeds was frequently not a precise boundary. I also learned to view seed age from two aspects, namely chronological and physiological, with physiological age being the most important." Others developed similar insights during the same period, roughly 1955-65, or even earlier, but Dr. Moore had, perhaps, the strongest influence on the present generations of seedsmen and seed technologists.

The concept of the dying seed provided the undergirding for development of present ideas, insights, and understandings relating to seed quality evaluation, deterioration, vigor, invigoration, enhancement, and so on. It is now well established that the dying of a seed is time dependent and involves the progressive impairment of vital systems until the seed loses its capacity for germination even under optimum conditions. In the context of function and utility, therefore, the relevant questions regarding the condition of a seed are whether it is live or dead, and, if live, how alive is it.

The dying of a seed begins at the time it attains physiological maturity and continues at a rate that is greatly influenced by inheritance, moisture content and temperature. Physiological aging, or seed dying, can be initiated and/or augmented by such agencies as as insects, e.g., stinkbug damage in soybeans, mechanical abuse, and phytotoxic chemicals.

The gradualness of the dying process in seeds is starkly revealed in the results of periodic tetrazolium (TZ) tests of a population of seeds. Indeed, the development of the TZ test was a watershed event in our understanding of seed quality. For Dr. Moore and many others, including myself, TZ test results were a revelation. The beginning, localization, spread, progress and terminus of the dying process are vividly manifest in the shades of red and white of the TZ test. TZ test practitioners not only read the degree of aliveness of seeds, but diagnose the probably inciting agent in drying seeds: bruises and fractures from mechanical abuse, thermal injury, insect damage, immaturity, even some minor element deficiencies.

The progress of dying in seeds, or their degree of aliveness, is manifest in many ways other than the results of TZ test. The slowing down of germination and growth as a seed dies is displayed in the results of germination tests. During the early decades of organized seed testing, analysts formulated and applied the concept of germination energy to assess the degree of aliveness of seed lots. In seed lots with a high degree of aliveness, germination is rapid and uniform, and growth of the seedlings is vigorous.

As a seed dies it becomes progressively more sensitive to microenvironmental conditions during germination. Put another way, it becomes less and less tolerant to environmental stress. Localized and spreading necrosis of seed tissue provides a foothold for molds which then establish themselves and accelerate the dying of the seed.

Many readers will have decided by now that for some reason I'm being coy, if not evasive. Degree of aliveness really is just a kinda quaint way of saying vigor, while seed dying is deterioration or loss of vigor. Fair enough, but I do feel that from time-to-time it is important to take a close look at the process (dying) rather than the consequence (loss of vigor) of a matter of great importance to all interested in seeds.

Factors Influencing the Rate of Seed Dying

The causes of seed dying operate at two levels. Seedsmen are more aware of and concerned about the external level causes because they can be controlled. The main external or externally influenced causes of seed dying are insects, diseases, high seed moisture, warm temperatures, mechanical damage, chemical injury, and storage molds. The external causes of seed death interact in just about all possible combinations to determine the rate of dying. Storage molds, for example, are an active cause only when seed moisture is high and the temperature is warm. Seed moisture, in turn, is related to relative humidity of the environment.

Many of the actions and procedures in seed quality control and conditioning have the objective of slowing down seed dying, through elimination or moderation of external causes. Timely harvest minimizes the exposure of the seed to rain (high moisture), wind and heat. Drying reduces seed moisture, while aeration reduces seed temperature. Use of appropriate handling systems and cautions operation minimizes mechanical damage, while sanitation and related measures prevent infestation with storage insects.

External causes of seed death sometimes couple with an internal level cause(s). In "The Dying Seed," Dr. Moore related how he gained his insight of "natural crushing" as an important cause of seed death in humid areas. Natural crushing is produced by mechanical stresses developed during differential intermittent swelling and shrinkage of the tissues of mature seed. The intermittent swelling is associated with alternating periods of rain and sunshine. Natural crushing was first recognized in seeds of soybeans, cotton, peanuts and other crops grown in humid areas. It does not occur or is inconsequential in cereal seeds, e.g. corn, wheat, because of differences in chemical makeup and embryo structure. It is of interest that in his discussion of natural crushing in soybean seeds, Dr. Moore touched on most of the factors still being researched. Natural crushing is more severe in early than in late maturing varieties, presumably due to warmer temperature. Seeds with light colored seed coats are more susceptible to natural crushing than those with dark colored seed coats. Wrinkling of the seed coat is a symptom if not an associated cause of crushing. Recent studies of black, pigmented and unpigmented soybean seeds and seeds with wrinkled seed coats with the scanning electron microscope corroborate many of Dr. Moore's early observations.

The internal level causes of seed death undoubtedly couple in various ways with the external level causes, thus, confounding cause and effect. Certainly they are only poorly understood. Lack of understanding, however, has never inhibited speculation. Speculation about the basic cause or causes of seed death, i.e., theories of seed deterioration, began early and continues to the present, because that's part, at least, of what science is about. A more practical reason for grappling with theories of seed dying is to gain a sufficient understanding to prevent or reverse it.

Theories of Seed Deterioration

In a 1965 article in the NewsLetter of the Association of Official Seed Analysts, J. G. Streeter posed this question: "Why does a seed, which is free from damage, unaffected by microorganisms, and stored in a desirable environment, lose its ability to germinate?" What Streeter was really asking in a round-about way was: Why aren't seeds immortal? Why do they die without apparent reason? He then went on to summarize the answers advanced by others because he was not the first to address the matter of seed mortality.

Nutritional Dysfunction

The nutritive function during germination of the starch, fats and oils and other materials stored in the endosperm and cotyledons of seeds has long been known in a general way. It is not surprising, therefore, that exhaustion of nutritive reserves and/or a change in their availability were among the first ideas about the dying of seeds. The idea of exhaustion of reserves might have been prompted by observations of the extreme loss of seed dry weight and certain "dying" during seed rots associated with pre-harvest weathering and severe storage mold activity. Since it is very evident, however, that most dead seeds are not rotten and have about the same weight as seeds still alive, the alternate idea of a change in the reserves from available to non-available was the more credible. Further, as the complex reserve nutrients have to be degraded to simpler forms before they become "available" to support germination, the problem was reduced to some dysfunction in the mechanisms for their breakdown into simpler, usable forms. There was not much evidence to implicate the reserve nutrients in deterioration of seeds at the time the ideas were advanced in the early 1900s and very little has been added since then. "Local starvation", Harrington's more recent (1960) version of the reserves depletion concept of seed deterioration, is much more intriguing and plausible. Harrington proposed that one cause of loss of viability in relatively high moisture seeds was exhaustion of readily available respiratory substate without replenishment from the complex reserves. The level of seed hydration while high enough to accelerate respiratory activity is not high enough for the hydrolytic breakdown of reserves into simpler forms and their translocation to the most active sites of respiration. The local starvation concept has not received the attention it deserves so its validity is not established. Certainly, it deserves rigorous examination especially in view of the prevailing idea that mobilization of the complex reserves is a post-germination event, or at least, is of post-germination significance, i.e., seedling growth.

In 1915 Crocker and Groves reported on a, "method of prophesying the life duration of seeds." The method was based on their idea that deterioration of seeds was the result of coagulation of proteins. They presented a mathematical model for predicting seed life based on the rate of "coagulation" of protein" at specific levels of temperature. Crocker later abandoned his protein coagulation theory because it was too general. Recent work, however, has established that proteins are broken down during aging and change in several other ways. The important thing in seed aging might be loss of the ability to preserve, activate or synthesize enzymes and structural proteins. Loss of biosynthetic capability is an especially attractive theory of seed deterioration.

Loss of Enzymic Activity

Many researchers have attempted to relate the dying of seed to the loss of activity of specific enzymes. Correlations - but not cause and effect relationships - were established between the activity of catalase, phenolase, glutamic acid decarboxylase, dehydrogenases and other enzymes, and germinability. The high degree of correlation in the case of the deghydrogenases combined with a simple type measurement to permit development of the widely used tetrazolium test for seed viability.

Genetic Damage

The two theories of seed dying that have greatest support are membrane impairment and genetic damage. The association of chromosomal changes with aging and severe death has been long and well established. DeVries, one of the giants in genetics, observed in 1901 that old seeds of a primrose species produced more abnormal plants than did fresh seed. Similar observations have since been recorded for a wide range of species. There seems no doubt that mutation frequency does increase with seed aging. In their classic review of the subject in 1956, D'Amato and Hoffmann-Ostenhof concluded that, "the most probable causes of loss of germinability are either lethal chromosome changes brought about by automutagenic substances accumulating during severe aging or a more general poisoning of the embryo due to accumulation of autotoxic substances." While the association of genetic damage with aging and death of seeds is well established, the cause and effect relationship is increasingly questioned. The genetic damage might be a consequence rather than a cause of deterioration.

Membrane Impairment

The most popular theory of seed deterioration is loss of membrane integrity or membrane impairment. There is abundant evidence that dysfunction in the reordering of membranes during rehydration or impairment during storage is closely associated with the processes of seed aging. Several types of vigor tests are based on measurements of the materials leached from seeds through "leaky" membranes. The causes of membrane damage appear to be lipid peroxidation as first proposed by Koostra and Harrington in 1969. In the model of lipid peroxidation advanced by Wilson and McDonald, lipids (fats and oils) in air dry seeds are autooxidized to hydroperoxides with liberation of free radicals. The membranes could be damaged directly by this process or by free radical transfer to other cellular components. The hydroperoxides would then be enzymatically broken down during the early stage of germination with generation of more free radicals and toxic secondary products both of which are implicated in seed death.

More Work Needed

Much more research is needed to develop a comprehensive, generally acceptable theory or model of seed deterioration. Such work is not only important from the standpoint of increased knowledge about deterioration of biological systems but also to provide the base for devising strategies to prevent or at least greatly reduce the rate of seed deterioration. For some time now, workers have been trying to slow down the dying of seeds through treatment with anti-oxidants, inhibitors of lipid peroxidation, and/or scavengers of free radicals. Even a moderate measure of success would require a lot of re-thinking about the mortality of seeds.