Economically sound decisions, questionable decisions, or wrong decisions—how do you use each in your business? Decisions as how best to bypass factors that downgrade seed quality or to upgrade seed of undesirable quality need to be sound, efficient, and timely. Extra profits are to be realized by sound and timely decisions. Financial adversities accompany unsound and untimely decisions.

Seed producers, seedsmen, managers of foundation seed programs, officials of certification programs, plant breeders, and other individuals or agencies that handle seed must constantly make decisions involving the hidden aspects of seed quality and of the opportunities to improve quality. The need for timely action oftentimes encourages costly, inappropriate decisions. The delay in awaiting growth test results and the inadequate information they provide frequently forces a person to take premature actions in handling the problems associated with the hidden but important aspects of seed quality.

The tetrazolium (TZ) test has been developed and refined as a rapid test to fulfill some of the basic needs for exposing and diagnosing many of the causes for inferior seed quality. The TZ test along with growth test results appear to answer most of the basic questions concerning causes for inferior seed quality. Each test is basically different. Neither test is as well understood as it should be understood. Each of us needs to spend more time trying to develop a more perfect understanding of the test rather than to spend our time trying to point out the imperfections of either test. The extra profits come from an understanding of the merits of a test, the information it reveals, and the use of the desired merits to resolve our seed quality problems.

Time on this occasion permits a discussion of only the TZ test and its use in quality control and for diagnosing causes for questionable or undesirable seed quality. During the past 20 years, I have never evaluated a seed by the TZ method without paying special attention to the possible causes for the disturbances present. Possible causes are often under study for several years before adequate segments of knowledge come together to provide the answer. Several disturbances are rather complex and conditioned by secondary factors. Some of these continue to remain unanswered.

I hear occasional comments that the use of the TZ test for diagnosing

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1/ Reprinted from the Proceedings 1973 Short Course for Seedsmen.

2/ Professor Emeritus of Crop Stands, North Carolina State University, Raleigh, N.C.
causes for embryo disturbance is strictly for experts. This is indeed not true. I have trained several people with a high school education to recognize many of the symptoms. It does take training and practice. We gain excellence in most of our activities by training and practice.

The TZ Test

A TZ test on a single sample of seed can provide within 12 to 24 hours the information needed for establishment of the potential germination percentage, soundness of germinable embryos, and evidence for establishment of causes for possible disturbances in quality. Test durations can be shortened to a few hours, or to less than an hour for special needs. Excessive shortening of testing time introduces some loss of accuracy of test results, which may still be acceptable.

The basis of a TZ test is the development of a red stain which permits the analyst to visibly observe the presence and location of sound, weak, and dead tissues. The nature of the patterns of staining permits a diagnosis of causes for the imperfections. Detailed instructions for conducting the test have been published by Grabe (1) and Moore (3,4,5).

Seed Preparation

If seeds are not already moist immediately prior to testing, it is usually desirable to moisten with water. The kinds and dryness of seeds determine whether the seeds can be moistened rapidly or slowly. Large-seeded legumes such as soybeans and snapbeans, especially when dry, tend to fracture readily and extensively when subjected to liquid water. The desirable slow absorption of water can be obtained by placement of seeds in a moistened, but not wet, paper towel or similar media.

Staining

Moist seeds of most kinds of small and large seeded legumes can be placed intact into the colorless TZ staining solution. Cutting or removal of seedcoats will hasten the rate of staining. The intact seedcoats of most grass seeds prevent the entrance of the staining solution. The larger grass seeds are usually bisected through the germs to expose the embryonic leaves and roots. Kinds of seeds considered too small for bisecting are usually punctured or cut near the germ.

The time of staining should be adequate to permit the distinction between normal, weak, and dry embryo tissue. The time can vary considerably without adversely influencing the results. Excessive staining time, however, is accompanied by tissue deterioration which prevents acceptable evaluation.

The rate of staining tends to double for each 10 F rise in temperature within a range of approximately 70 to 100 F. The duration of staining must be shortened at a higher temperature so as to avoid excessive deterioration.
The testing solution is prepared by the addition of tetrazolium salt to tap water. Solution strength can vary from approximately 0.1 to 1.0%. The TZ salt costs about 40¢/gram and can be obtained from Nutritional Biochemicals Corp., 26201 Miles Road, Cleveland, Ohio 44128, as well as from several other chemical companies.

Causes of Seed Deterioration

Common causes for seed deterioration vary with the kind of crops, region of production, and methods of harvesting and processing. Common causes include mechanical injury, water damage, aging, heating, freeze injury, diseases, and insects. Accelerated aging tends to accompany and may even conceal some of the other kinds of trouble.

Mechanical Injury

Disturbances resulting from mechanical injury may be external or internal—usually both. The internal injuries, which are usually most prevalent, may be revealed as fractures or bruised tissues, or both. Embryos that are damaged when excessively dry may show normal staining qualities even to the edges of the fractures. When tissues are moist at the time of injury, the areas impacted tend to develop a darker than normal red color immediately after injury. With time, the crushed cells gradually die and are no longer capable of staining.

Water Damage

Mature seeds, especially of large seeded legumes and cotton, are initially and subsequently damaged in many ways by exposure to alternate wetting and drying prior to harvest and during storage. The initial damage in turn promotes accelerated aging and infection. The symptoms are usually associated with various levels of aging within and surrounding the initially damaged areas.

Certain types of water damaged symptoms can be confused with injuries resulting from mechanical fractures or bruises. A trained analyst, however, can usually make correct diagnoses for the majority of seed within a sample.

Water damage of the type being brought into focus on this occasion is especially prevalent in snapbeans, cowpeas, soybeans, lupines, etc. Two general types of symptoms occur. One type involves the obvious fracturing of embryonic tissues, and the other involves deterioration of localized areas of embryonic tissues. Both types of damage may occur within the same embryo.

Fracturing is prevalent in production regions where rapid and extensive drying of mature seeds occurs between the periods of occasional rainfall or other forms of high humidity. The damage results from stresses established within embryo structures by rapid localized absorption of free water. Fractures frequently occur within radicles and at the attachment of cotyledons to the embryonic axis. The nature of the damage varies from different varieties, crops, weather conditions, etc.
In snapbeans, the epicotyl or the plumule tend to fracture rather extensively. In soybeans, fracturing tends to be more extensive within the radicle with very little fracturing of the epicotyl and essentially no fracturing of the plumule.

A second type of water damage is caused by alternate wetting and expansion, and drying and shrinkage of seedcoats with exposure of mature or nearly mature seeds to alternate wetting and drying. The nature of the disturbance in large seeded legumes is somewhat as follows. Upon moistening, the seedcoat tends to expand rapidly and irregularly and becomes folded like an accordion. The innermost folds come into contact with localized less moist surfaces of the embryo. The rapid movement of free water from the coat into the adjoining embryonic cells cause various types of disturbances among and within cells. The disturbed cells are first weakened and later die. The phenomena was earlier reported as natural crushing by Moore (7), which, in view of today's knowledge, may need a more appropriate terminology. Additional insights into the nature of this disturbance are likely provided by Iljin (2) and Stadelman (10) in studies with other types of tissues concerning plasmolysis-deplasmolysis phenomena.

Further insights into the nature of water damage, including hollow hearts of peas, are found to be in articles by Moore (6,9). Such insights resulted from intensive studies with tetrazolium staining, which most seed physiologists have not pursued.

Aging

Aging needs to be considered from two viewpoints, namely chronological and physiological. Chronological age refers to the lapse of time after a given lot of seed matured. Physiological age refers to the degree to which embryo tissues have advanced as a result of aging processes. Such processes are accelerated by high temperatures, high moisture, injuries, and genetic composition. The relationship between chronological and physiological aging is not very predictable without a knowledge of environmental factors to which seeds have been subjected. In TZ testing, we are mainly concerned with the physiological type of aging.

It is desirable to consider two general types of accelerated deterioration associated with localized injured tissues. The other type represents a slower form of deterioration associated with masses of non-injured tissues that are not in close contact with obviously injured tissues.

Accelerated aging is commonly associated with mechanical injuries and weather damage. Centers of damaged areas, if stained before much additional deterioration or aging has occurred, tend to stain dark red. With additional time, the severely injured areas of tissue tend to become dead and fail to stain. A border of deeply stained tissue surrounding a necrotic area represents rapidly aging tissue. With time, the inner layers of cells of the border become dead and the outer
periphery of the necrotic zone enlarges by transformation of normal cells into a weakened condition. The extensiveness of the dead and dying tissues reflects the extent of physiological aging of restricted areas.

General aging tends to advance along with localized aging but at a much slower rate. Theoretically, the general aging symptoms progress rather uniformly with initially non-injured embryo tissues. In practice, however, this high level of uniformity of aging is seldom observed. Certain tissues assumed to be non-injured still tend to age more readily than other areas. These areas likely reflect minor disturbances from slight pressures, unequal moisture uptake and release, unequal exchange of air, etc.

In TZ tests, the aged tissues tend to be flaccid and pale red to white in color. Different degrees of aging are reflected. The greyish red color commonly seen on cut surfaces of corn and small grains is believed to be due to a reaction between TZ and sulfur bonds of partially denatured proteins.

Heat Damage

The storage of moist seeds without adequate ventilation to remove heat tends to give rise to blurredness of tissues and a brownish red stain as observed in the TZ test. Damage is often more intense on some structures than on other structures. Radicle tips and plumules of dicots are usually rather sensitive to early stages of heat damage.

Excessively high temperatures in drying cause injuries that also can be readily detected by TZ tests. In case of corn and small grain, the embryonic tissues tend to remain flaccid and to develop an abnormal color.

Freeze Injury

Freeze injury varies considerably in severity. Light amounts of freeze injury in corn, for example, may cause a slightly darker red than normal stain. Severe freeze to high moisture corn tends to kill the embryo. Intermediate levels of damage are reflected by blurredness of tissues and a tendency for a greyish or purplish red stain.

Freeze injury in soybeans tends to produce a bluish red "liquid logged" condition. The damaged area tends to be localized in sections of the seed where free water was accumulated at time of freeze. Soybeans are rather resistant to freezing but can be damaged extensively.

Diseases

Diseases that cause most disturbance in germination tests are saprophytic. The fungi generally require dead or weak tissues as infection centers. Once established, they gradually weaken, kill, and move into nearby tissues. Mechanical and weather damaged tissues serve as commonly encountered infection centers.
In TZ tests, the diseased tissues tend to be soft and mushy and sometimes brownish red. The areas tend to be circular and bordered by a deeper-than-normal red color.

**Insect Damage**

Damage by chewing insects is obvious. Damage done to soybeans by a piercing insect, the stink bug, deserves special comment. The seeds are damaged while immature. The damaged area tends to be sunken and spongy. The surface tissue on the embryos appears drawn. A small puncture scar can be noted near the center of the damaged area. The tissue stains a dark red color or may fail to stain if sufficient deterioration has occurred.

**Summary**

The TZ test is unsurpassed in the timely diagnosis and evaluation of seed quality problems. The test is gradually gaining acceptance in the diagnosis of the presence and seriousness of injuries resulting from mechanical impacts, field weathering, aging, heat, freeze, diseases, and insects. It is especially useful in quality control programs for guiding economically sound decisions in reference to harvesting, processing, blending, storing, treating, marketing, and carryover problems and opportunities.

The tetrazolium test, along with the standard growth test, will expose nearly all commonly encountered seed quality problems.

The basic principles of TZ testing can be grasped rather quickly from a few hours of instruction by a competent analyst. Considerable experience is needed for a high level of excellence in its use. College training is not required.
REFERENCES


