1-1-1966

Tetrazolium Best Method for Evaluating Seed Life

R. P. Moore

Dr. Moore Firm In Belief...

Tetrazolium Best Method For Evaluating Seed Life

...Advises Reconsideration

By Dr. R. P. Moore

Professor, Research, Crop Stands
N. C. State University
Raleigh, N. C.

EDITOR'S NOTE: Alarmed over the talent and money being wasted on new methods of seed testing, Dr. Moore expounded on his faith in tetrazolium testing at the recent meeting of the American Society of Agronomy in Stillwater, Okla. Sketching the history of the chemical, he ended his talk by urging a new look at tetrazolium. Here then is his heretofore unpublished research paper.

Information from early studies for development of quick tetrazolium test, largely obtained from German, Japanese and Russian publications, and from personal communications, should help keep us away from the pitfalls discovered by our predecessors.

Information culminating in the tetrazolium test came from investigations in bacteriology, chemistry and medicine, as well as from plant physiology and seed testing laboratories. Even tanneries of wild animal hides in Germany and the frequent outbreaks of anthrax from shipments of infected raw hides made a contribution by creating needs for new and safe vaccines. Vital stains, as early as 1900, were found useful for checking these vaccines for live bacteria.

When viewed from today's knowledge, the early quick tests were characterized by many false assumptions and missing segments of essential knowledge that were gradually brought into focus with attainment of new knowledge. Most studies were made with very few seed lots, and usually with samples that were conditioned artificially. Common misconceptions seemed to be the belief that a seed had to be either dead or alive. For a long time, researchers tended to avoid the necessity of studying individual seeds, and for even a longer period of time they ignored recognizing the importance of evaluating the individual structures of individual seeds. This appears strange, yet even in 1966 these oversights are still leading many investigators astray.

The earliest test included – cutting, fluxing, H_2SO_4, alkaline silver, methylene blue, malachite green, dinitrobenzol and mass measurements of diastase, peroxidase, starch, heat of respiration, osmosis, leachates, electrical currents, etc. Frequently, all seeds within a sample were pulverized *en masse* and chemically evaluated in a beaker, a typical method for a biochemist.

Most of the early methods were found to be hopefully successful by a few investigators and for certain specially selected seed samples. Many exceptions were pointed out by other investigators. With time, as knowledge of seed and testing methods gradually became perfected, an increasing number of plant physiologists began to recognize unmistakably the potential usefulness of vital stains and started directing their studies in this direction.

Noticeable improvements in seed evaluation by use of vital stains were evident by 1920. A considerable advancement occurred, however, in 1922 when Dr. Turina of Yougoslavia discovered that living plant cells were capable of reducing the colorless salts of selenium and tellurium to the reddish or blackish color of the pure chemicals.

Dr. Neljubow of Russia who was unmindful of Turina's work, but who knew of the use of selenium and tellurium salts in 1900 for staining bacteria, was to establish the next milestone in biochemical seed testing. In 1925 he published a historical review of the use of various vital stains in bacteriology in which he also critically evaluated early attempts by seed physiologists to resolve the problems that were hindering the success of different rapid methods for seed evaluation. He attempted to find vital stains that were nontoxic so that the stained seeds could also be studied in growth tests. After trying juices of beets and flower petals and many commercial stains he reported the most success with Indigo carmine. Numerous staining problems, however, were yet to be resolved. A better stain than Indigo carmine was needed. Neljubow indeed was approaching success but died in about 1928. His bacteriologically trained superior, Dr. Issatschenko and co-workers, were able to do little more than re-evaluate Neljubow's work and to publish a few details in procedure and to popularize Neljubow's achievement with Indigo carmine.

Although Neljubow's studies were outstanding as published, they became even of greater significance by stimulating Dr. Lakon of Germany, and Dr. Hasegawa of Japan to join the battle of ideas involved in the further development of quick tests.

Lakon's first interest in quick tests was revealed in 1928 in an article that provided a brief appraisal of the various methods proposed for quick tests. He concluded that methods involving the measurements of pulverized or intact seed masses, such as release of CO_2 and leachates, and activities of diastase, catalyse, dehydrogenase, etc., were hopeless. He also concluded that the tests as proposed for individual seeds, such as H_2SO_4, leachates, Indigo carmine, oxidase thus far had only limited value. To him the staining method appeared most promising, but success on the whole was still slightly out of reach.

Lakon's views published in 1928 take on special significance when considered along with his keen insight into seed life as revealed in a 1918 paper and series of paper starting in 1939.

By 1930 achievements were starting to come rather rapidly. Hasegawa of Japan who had spent 10 years studying enzyme activities in tree seeds entered the race in 1931. His aim was to develop a rapid practical test for tree seeds. After re-evaluating the more promising suggestion of other workers, he readily concluded that too many exceptions were being encountered. He was first to find that the salts of selenium and tellurium were expecially promising and proceeded to develop the use of tellurium for practical use.

Hasegawa's exceptional thoroughness of approach and comprehension of the over-all aspects of the problems associated with seed evaluation closely paralleled that of Neljubow. Hasegawa's early articles were published in Japanese and, therefore, not readily accessible to physiologists in western Europe. Communications were established in 1935 when Hasegawa made a trip to Europe and went to Eberswalde, Germany, to visit with a Dr. W. Schmidt about his enzyme studies with tree seeds. On this occasion Hasegawa missed Schmidt but met a Dr. Eidmann who became vitally interested in Dr. Hasegawa's studies with selenium and tellurium salts for staining embryos.

Hasegawa at first was reluctant to reveal certain details of his testing procedures to Eidmann, largely because Hasegawa's station in Japan had applied for a patent so as to control quality of testing paper and testing prices. Eidmann overcame this reluctance, however, by wining and dining Hasegawa in his home convincing him that the patent applied to Japan, not Germany.

Within 10 days Eidman obtained chemicals that were unavailable to Hasegawa because of wartime restrictions. Upon returning to Eberswalde after a 10-day absence, Hasegawa was disturbed to learn that Eidmann had started publicizing the Eidmann Biochemical Seed Testing Method. Hasegawa insisted that this was the Hasegawa method. Schmidt was soon to agree with Hasegawa, and very shortly Schmidt and Eidmann were blasting away at one another in a series of professional articles in a weekly magazine. Eidmann's viewpoints and the literary charges and counter charges were especially constructive and did much to strengthen and popularize the use of selenium in seed evaluation. In the meantime, Eidmann lost his job in about 1938 and went to Western Germany where he never again concerned himself with biochemical seed testing. As a loser, he became a popular hero in the history of quick tests.

Even though both Hasegawa and Eidmann made significant contributions in the field of biochemical testing, something vital to success was still missing. Too many unexplainable discrepancies were arising between chemical and growth tests.

Lakon had now become increasingly concerned about the fallacies perpetuated by Neljubow, Hasegawa, Eidmann, *et al* in assuming that a certain relative amount of the embryo should be stained for a seed to to be considered capable of germinating. Lakon also recognized the potentialities of a colorless vital stain, as selenium, as a reasearch tool for digging more deeply into questions about seed physiology that he had raised in 1918 concerning reasons for differences in germination potential, germination tendency, germination percentage, etc.

After being challenged by Eidmann's convincing reports about selenium testing, Lakon was soon to establish studies for determining the spread of death within dying seeds. He felt that if he could determine how a seed dies, that he could make Eidmann's selenium method much more reliable.

With Meljubow dead, Eidmann fired, and Hasegawa's station soon to be bombed-out, Lakon was destined to be a winner. Lakon published a comprehensive paper in 1939 involving selenium staining studies, and concerning the loss of germination capacity. This was followed in 1940 by another equally outstanding paper concerning the topographical selenium method. He had at last provided the major missing links in earlier studies – the need for specific embryo parts being alive. This he called his topographical method.

Lakon quickly realized the poisonous characteristics and noxious odor of selenium and started searching for a similar but non-poisonous compound. A chemist at Heidelberg called Lakon's attention to tetrazolium salts that had been used in research studies concerning the possible use of invert soaps as bactericides. Lakon obtained several of these salts from which he chose the 2, 3, 5, Triphenyltetrazolium as being most suitable.

He immediately applied his topographical method to the new staining material, and in 1942 published a paper concerning the topographical determination of the germination capacity of cereal seeds by tetrazolium salts and another paper concerning the use of the same test for corn. From then on and especially after retirement in 1950 the number of his publications increased rapidly as he adapted the method to first one and then another class of seeds.

We in America first learned of Lakon's work in 1945. This was shortly after personnel of the Supreme Headquarters, Allied Expeditionary Force visited Lakon's laboratory and wrote ---"The results of this work will be of tremendous importance in regard to improved crop production in the U. S.---,every encouragement and assistance should be given to Dr. Lakon for the continuation of his important work."

Space does not permit details concerning Lakon's 1950 trip to America --his disappointment in not being able to introduce his topographic tetrazolium method --his problems in getting suitable tetrazolium salts produced --his strong personality and impatience with other seed physiologists who failed to grasp the new and significant insights into seed life that tetrazolium studies had so clearly opened up to him.

These and many other insights have greatly strenghtened my faith in tetrazolium testing and in the firmness of the foundation upon which the method has been developed and promoted.

With this background I am concerned about the personal talents and money that are still being apparently wasted by the discovery of so-called "new" methods that were repeatedly tried and discarded as men have searched for at least 75 years for a suitable rapid method for evaluating seed life.

I strongly urge that anyone attempting to evaluate seed especially in mass would take time out to gain a tetrazolium viewpoint of characteristics that make seeds weak or non-germinative. With this knowledge one can be guided around pitfalls that hindered early testing programs and that remain open to receive seed investigators that have yet to take a really close look and the individual parts of an individual seed.