

Standardization of Seed Vigor Tests

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Introduction

The last 30 years have led to increasing dissatisfaction by seed producers and consumers with the standard germination test as the sole criterion of seed performance potential presented on a seed label. As a result, new seed testing techniques have been formulated known as seed vigor tests that provide more sensitive measures of seed quality. The ISTA and AOSA Vigor Test Committees have been at the forefront of this development. These Committees and their respective members represent the authorities in vigor test development. They have evaluated an array of proposed vigor tests, provided protocols for those that are most useful, sponsored seed vigor testing workshops, and published educational materials such as their respective Seed Vigor Testing Handbooks that define the strengths and limitations of each vigor test. This leadership has produced important results. In 1978, 52% of the seed testing laboratories in the United States were conducting vigor tests. In 1983, this increased to 63% and 1990, 75% of the seed testing laboratories were using one or more vigor tests (McDonald 1994).

Why do we test seeds for vigor? This question is seldom asked because there is an intuitive answer: “Because we have to know how they will perform in the field.” But there are even more subtle rationales for testing seed vigor than this. We test seeds because “a seed lot is composed of a population of individual seed units; each possessing its own distinct capability to produce a mature plant. A seed vigor test is an analytical procedure to evaluate seed vigor under standardized conditions. It enables a seed producer to determine and compare the vigor of a seed lot before it is marketed” (McDonald 1988). Thus, we test seeds not only to determine how they will perform before they are actually planted, but also to provide exact standards for testing in order that the results are reproducible among laboratories testing the same seed lot; a process known as standardization.

General agreement exists that seed vigor testing is a needed component of seed quality analysis. Yet, this information is still neither required nor found on a seed label. The purpose of this presentation is to focus on those factors limiting standardization of vigor tests and to identify areas that may speed this process. The development of useful seed vigor tests likely will take many years before completion. Not to be forgotten is the fact that the first seed legislation was passed in Berne, Switzerland in 1816. Over 100 years elapsed before the first Rules for Testing Seeds were published. The evolution of seed vigor testing too has encountered many social and technical challenges that have delayed its acceptance.

Social Challenges

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Attitudes

Among the central questions concerning seed vigor is, “Who are the users of such information?” Certainly, major seed companies benefit from seed vigor testing and most have developed and now conduct seed vigor tests on a routine basis. There are a variety of ways that seed vigor test information is important to a seed company. Vigor tests can monitor seed quality through every seed production phase from harvesting, storage, conditioning, bagging, and planting. They enable adverse practices to be readily detected and corrective action taken. In some cases, such information can identify additional measures needed such as seed treatment (e.g., fungicides or seed enhancements). Seed vigor tests also are used in inventory management. Many types of valuable hybrid seed, for example, require important decisions on storage to minimize space allocation, reduce costs, and still provide an acceptable product to the consumer. Vigor tests can identify which seed lots are most likely to retain quality during long-term storage. They also assist seed companies in establishing minimal seed quality levels for marketed seeds. These important attributes have resulted in rapid acceptance of seed vigor tests as essential quality control measures by the seed industry.

While the seed industry has been quick to incorporate seed vigor testing into their quality control programs, similar acceptance of this information by the seed consumer has yet to be attained. There can be little doubt that the seed consumer can benefit from seed vigor information in various ways. It would, for example, help the consumer decide on the fair price of a seed lot. Beyond purchase price, however, the seed consumer could use vigor test information to determine how early in the season to plant, the quantity of seeds to use for satisfactory emergence, the expected uniformity of stand for subsequent secondary tillage and/or pesticide operations, the conditions of environmental stress such as cold, drought, soil compaction, etc., that might be tolerated, and how plentiful and uniform a harvest could be anticipated.

With these important advantages, why haven't seed consumers routinely requested or even demanded that vigor test information be presented on a seed label? One principal reason is that the seed industry has adopted the attitude that seed vigor tests are not sufficiently standardized between and among seed laboratories (see pp. 34-35, AOSA Vigor Testing Handbook) so reproducible results can be obtained. If this is true, seed lots moving in interstate or international commerce that are tested for vigor at the departing and receiving stations may produce differing results which makes marketing difficult and litigation likely.

Another important reason is the attitude among seed vigor test research leaders that the development and standardization of vigor test must be done cautiously. Too hasty an adoption of vigor tests and subsequent challenges in legal courts could be detrimental to vigor test credibility and do more harm than good. In an attempt to speed the process of vigor test adoption, the AOSA Vigor Test Committee has partitioned vigor tests into two categories: suggested and recommended tests. Suggested vigor tests are those that correlate with seed vigor and offer promise as standardized vigor tests. Recommended vigor tests are those that have been critically evaluated for determining seed vigor and are considered standardized. An example of a recommended vigor test is the accelerated ageing test.

Another prevailing attitude is that vigor tests should be conducted using the same general technical approaches applied to germination testing. For example, most seed analysts would prefer that vigor tests

be conducted with the same equipment and temperature and evaluation times employed in the conduct of a germination test. But vigor testing is a more sensitive measure of seed quality and it stands to reason that seed vigor tests must, therefore, require more rigorous control of test variable and interpretation criteria. To the seed analyst, seed vigor testing must be recognized as a more precise and challenging measure of seed quality.

Technical Challenges

It is clear that numerous social issues still confront seed vigor testing. Beyond these issues, other technical challenges remain before vigor testing achieves the same acceptance as germination testing. These issues include vigor test requirements, variables in vigor testing, and presentation and interpretation of vigor test results.

Vigor Test Requirements

Vigor tests are more sensitive measures of seed quality than the standard germination test. The standard germination test result is the product of a “first” and “final” count. The first count is the time when most seeds germinate and the seedlings are moved before they become too unwieldy. The second count provides the opportunity for slow to germinate seeds to demonstrate their ability to germinate. The final count is conducted at a “safe” time that provides sufficient opportunity for even the weakest seeds to germinate. Seed vigor testing is not afforded this luxury. Most seed vigor tests monitor some aspect of seedling or biochemical growth. It is important to differentiate between weak and strong seeds at the earliest possible moment, so timing of seed/seedling evaluations must be precise. When 72 hours is recommended, it should not be 76 hours. For example, the accelerated ageing test protocol appropriately emphasizes that not more than one hour should elapse beyond the recommended accelerated ageing period or the test results may be invalid. The flexibility in timing enjoyed by analysts in conducting a standard germination test serves as a poor model for seed vigor test analyses.

Similarly, environmental factors such as moisture and temperature must be precisely controlled in vigor tests because of their immense impact on the rate of seedling growth. It is interesting that no specifications for either the type of substrata or the amount of water used have been indicated for most seed vigor tests. Yet, both factors strongly influence the availability of water to the imbibing seed and its subsequent rate of growth. We know, of course, that some laboratories prefer to conduct their germination tests “drier” or “wetter” than others. Vigor tests that use a component of seedling growth as a parameter of seed vigor should define the level of moisture used in the seed test.

Temperature requirements also influence the rate of seedling growth. In germination testing, a $\pm 1^{\circ}\text{C}$ differential in seed germination is permitted. In some cases, seed analysts conduct germination tests at either constant or even alternating temperatures. But, we know that types of germinators differ among laboratories. Some are wet, others dry. Some recover more rapidly from alternating temperature regimes and still others are better able to maintain a $\pm 1^{\circ}\text{C}$. When conducting vigor tests, seed analysts must understand the importance of uniform temperature maintenance throughout the duration of the test.

Explanations for differences in vigor test results, particularly among laboratories, should begin with questions regarding the quality of germination equipment and assurance of accurate temperatures throughout the test. Perhaps the best example of appropriate emphasis on temperature is provided for the accelerated ageing test where temperatures are required to be within tolerances of $\pm 0.3^{\circ}\text{C}$ and

specific types of commercial equipment are recommended for the conduct of the test (ISTA 1995). These precise descriptions are excellent models for other vigor test protocols.

Variables in Vigor Testing

The development of acceptable vigor tests is a challenging goal. Unlike germination testing, there is no one standard test against which the merits of a vigor test can be judged. Part of this difficulty arises from the perception that no universal seed vigor test exists. Presently, most vigor tests available are recommended for a specific crop. It seems that one central theme should be to reduce the range of vigor tests and focus standardization efforts on those that appear to have the broadest crop application. Next, the optimum times and procedures for each crop need to be determined and specified.

Beyond these technical problems, there are also a number of variables that affect seed vigor tests but which have yet to be adequately considered in vigor test result interpretation.

Seed Size

Seed lots vary in seed size and this can have an effect on vigor test results. In some instances, accommodations have been made for the effect of seed size in modifying vigor test results. For example, bigger seeds would be expected to leak more electrolytes than smaller seeds even though they may be more mature and vigorous. As a result, conductivity results are presented on a seed weight rather than a per seed basis. Similarly, smaller seeds because of their greater surface area to volume ratio absorb water at a greater rate during an accelerated ageing test than larger seeds. To account for this, a certain weight of seed is specified for placement in the inner ageing chamber rather than a specified number of seeds. It should also be emphasized that many vigor tests monitor some aspect of speed or rate of germination. Smaller seeds tend to complete imbibition and initiate radicle protrusion before larger seeds. These examples demonstrate that seed size modifies test results that are separate from an assessment of seed vigor. A consideration of any new vigor test should ensure that seed size does not bias test results.

Seed Treatment

Seeds are treated to reduce the invasion of pathogens following planting. But, how should seeds be tested for vigor: in the treated or untreated condition? Some argue that seeds which benefit most from seed treatment are those which are low in seed vigor. In this example, seed treatments “artificially” enhance seed vigor of seed lots inherently low in seed vigor. According to these advocates, a true test of seed vigor can occur only with untreated seed. Others contend that seeds should be tested for vigor in the same way that they would be planted in the field. After all, one important component of a seed vigor test is its indication of a seed lot’s field performance potential.

Surprisingly few vigor tests provide guidelines on seed treatment. The conductivity test recommends that seed treatments be removed prior to test. Other tests (cold test, accelerated ageing test) suggest that the seed be tested in the way that it is received in the laboratory. One thing is certain, seed treatments can affect the results of a vigor test, particularly when comparing the results of a treated seed lot with those of an untreated seed lot. As an example, the cold test was originally developed to test the efficacy of seed treatments. Any proposed vigor test should provide guidelines concerning seed treatments. This recommendation becomes more important as more and more seeds are either treated by pelleting, film

coatings or physiological enhancements such as priming and biocontrol.

Seed Dormancy

Many important crop seeds do not have dormancy because this trait can be detrimental to stand establishment and has been eliminated through breeding. However, a study of germination testing procedures in the Rules for Testing Seeds reveals that dormancy is still an important problem in seed testing of numerous crops. But, how should dormant seeds be evaluated by a seed vigor test? Since most vigor tests rely on some measure of seedling growth, does this mean that dormant seeds are low in vigor? What would be their level of vigor once dormancy was broken? For example, the conductivity test determines leakage from imbibing legume seeds. In some years, hardseededness (impermeability to water) in legumes is expressed and these seeds would not leak electrolytes, thus lowering their overall conductivity value. Another approach is to ensure that dormant seeds are reported in germination test results with the recognition that these seeds will not contribute to vigor testing values. Whatever the best answer, guidelines for the treatment of seed dormancy in vigor testing need to be provided.

Vigor Test Design

There are also variables that influence interpretation of results based on the design of the vigor test. For example, most vigor tests evaluate individual seeds/seedlings and then provide a composite value such as a percentage for the seed lot. Others, such as the conductivity test, are a bulk test where all seeds are treated in the same way at the same time and the results expressed as an average value for all seeds. This vigor test design has merit because it is more rapid and less expensive to conduct than individual seed analyses. However, the results must be interpreted with caution. The general understanding of a conductivity results is that it represents an average value applied to each seed. However, it is also possible that there may be one bad “leaker” in the seed lot while the remaining seeds are excellent. This bad “leaker” would increase the conductivity reading and the result would suggest that all seeds were average when, in fact, the seed lot is of overall excellent quality.

Another approach to vigor test design is based on recognition that most vigor tests determine specific facets of seed quality. For example, the accelerated ageing test provides an indication of the storability of a seed lot while the conductivity test evaluates membrane integrity. Both components are important determinants of seed vigor. As a consequence, it has been proposed that greater information concerning seed quality could be acquired by conducting a battery of seed vigor tests and summarizing the results as a single vigor test index. This approach is sound but it is difficult to successfully implement. One reason is that it is uncertain whether equal weight should be given to the values provided by each of the vigor tests or whether certain test values provide more vigor test information than others. In one case, the user may decide that cold test results provide more sensitive vigor test information than conductivity results. But, if this is so, by how much? Another reason why vigor test indices have not become more common is related to the cost: benefit ratio. The increased vigor test information provided by additional vigor test may not be sufficient to warrant the increased cost and time required to generate the data.

Time Results are Valid

Seldom is there a discourse about the length of time that vigor test results can be considered valid, but this dialogue should begin. The U.S. Federal Seed Act mandates that seeds in open storage must be retested for germination after five months, or 24 months when hermetically sealed. Because vigor tests are more sensitive determinants of seed quality, it is assumed that retest intervals for vigor would be

shorter than for germination. But, by how much? Are certain vigor tests better able to determine seed vigor than others? If so, would this time frame vary according to the test result? What influence would seed storage environment have on the validity of vigor test results over time?

Use of Standards

Neither the ISTA nor AOSA Vigor Test Handbooks advocate the need for standards when interpreting the results of a vigor test. This quality control process needs to be encouraged when conducting vigor tests for the following two reasons. First, a seed testing laboratory should have a standard seed lot for which vigor test values are known that is either routinely or anonymously introduced into the testing regime. Because seed vigor testing requires precise environments and analyst interpretations, these standards identify when test results may be altered or “out of tolerance” due to conditions external to the seed.

Second, standards can also be employed in comparing results among laboratories for difficult to standardize tests. For example, the use of local soils in a cold test makes comparison of test results among laboratories difficult because soils vary in their pathogen levels and water holding capacities. Both of these factors have a major impact on cold test results. However, if the laboratories comparing seed lots use the same seed standard, they might be able to express the results on a percentage basis relative to the standard. Thus, a seed lot may have a cold test result of 35% and the standard may be 45% at laboratory A. At laboratory B, the cold test may be less stressful and produce results of 70% for the seed lot and 90% for the standard. While these absolute values are different, they are the same when computed on the basis of the standard seed lot (laboratory A: $35/45 = 0.77$; laboratory B: $70/90 = 0.77$).

Presentation and Interpretation of Vigor Test Results

Presentation

Still unresolved is how to best present seed vigor test results. Tests, such as the cold and accelerated ageing tests, that provide results on a percentage basis, lend themselves to immediate acceptability by the seed consumer because of their similarity with germination test results. Yet other tests are just as valuable but the presentation of results is more problematic. For example, the tetrazolium test is one of the most useful seed vigor tests, but how should the colorful, topographic staining patterns be presented to the consumer in a meaningful way? The conductivity test, too, is an important test of seed vigor for many crops. However, the data are presented in $\mu\text{S}/\text{cm}/\text{g}$ seed. To the uneducated consumer, what does that value mean, particularly as it applies to field emergence? More importantly, since there is an inverse relationship with seed quality, higher readings mean poor quality seeds which is a conclusion opposite from what most people would expect. Some have suggested that the interpretation of results would be assisted by providing arbitrary categories. Readings below 20 $\mu\text{S}/\text{cm}/\text{g}$ are acceptable, readings above 40 $\mu\text{S}/\text{cm}/\text{g}$ are unacceptable, and those in between are considered marginal. At first glance, this appears an acceptable alternative, but how does one classify a seed lot with a reading of 19.9 $\mu\text{S}/\text{cm}/\text{g}$ and another seed lot with a reading of 20.1 $\mu\text{S}/\text{cm}/\text{g}$; particularly in cases of litigation? Are these lots significantly different in quality as the arbitrary classification would lead one to believe?

Interpretation

This example provides the forum necessary to initiate a discussion of interpretation of vigor test results.

Inevitably, as any new test is formulated, the consumer using the information asks what the data mean. In seed vigor testing, this question is often related to some component of field performance and we have been guilty of this association. I contend that it is not the responsibility of seed analysts to interpret vigor test results and we exceed our role when we speculate what the values of any particular test mean in terms of subsequent seed performance. The seed analyst's role is to accurately present the test data and allow the consumer the responsibility of interpreting whether the seed lot is of acceptable quality based on his/her own standard(s): a time-honored process known as a *caveat emptor*.

Another concern in interpretation is the implied association of vigor test results with field performance. For example, let's take a seed lot with a standard germination test of 90% and a cold test of 65%. Individuals have concluded that this seed lot would produce a stand of 90% under ideal conditions and 65% under stress conditions. Let me caution such individuals that seed vigor test results do not provide a forecast of stand emergence! At most, a seed vigor test allows the consumer the opportunity to determine that one seed lot is superior to another. The degree of this superiority is determined in the field at the time the environmental stress is experienced for that particular year. In some years, the stress may be so severe that no seeds emerge, even for the most vigorous seed lot. In others, seed vigor tests may actually correlate with stand establishment. The important point is that seed analysts should avoid predicting or forecasting field performance of a seed lot based on vigor test results. It can be said that one seed lot will be better than another. How much better depends on the particular environment experienced at planting.

Both of these examples illustrate the importance of seed vigor education for the seed consumer. Our enthusiasm for extolling the merits of vigor testing can lead us into unfortunate overstatement of its virtues. This may make the consumer feel that he/she is getting more information concerning the quality of the seed than can realistically be expected. There is little doubt that vigor testing is useful in a variety of ways to the seed consumer. Let us be secure enough in these attributes without overstating them. In this context, the ISTA and AOSA Vigor Testing Committees are applauded for providing the educational leadership for appropriate presentation and interpretation of vigor test results.

Consensus and Unification

It should be clear that many vigor testing issues still require clarification and solution before standardization of vigor tests is achieved. But, where do we go from here and how can we most efficiently resolve these issues and supply the seed consumer with this valuable seed quality information? Some have contended that two groups, ISTA and AOSA, working on the same problem and developing differing tests and interpretations have resulted in a duplication of effort which has not been in the best interest of vigor testing. I advocate that this is not so. These two progressive groups have produced the creative, competitive environment necessary for the most rapid evolution of seed vigor testing while simultaneously providing the global view needed for specific vigor tests. These independent drives by both groups culminated in the production of respective Seed Vigor Testing Handbooks that, while similar, are different because of their separate heritage.

The two Committees are applauded for their leadership in seed vigor testing. Now, however, appears to be an appropriate time for consensus and unification of these diverse views. At this point, it seems that

competition between the two Committees and confusion about which Handbook is more authoritative or better can be detrimental to vigor testing. More importantly, further delay in advancing seed vigor testing is not helpful. The world is “smaller” with international shipment of seed increasingly common and necessary. The ideas and concepts of seed vigor testing as well as protocols for the most accepted seed vigor tests have been captured in the respective Handbooks. These must be coalesced into a single, authoritative source. Leaders in seed vigor testing must unite as a single body so that the seed consumer knows who can provide acceptable information on the merits of seed vigor testing.

At this consensus and unification occurs, we must still wrestle with the vagaries of seed vigor testing. This discussion has emphasized how vigor testing is a more sensitive measure of seed quality and requires more sophistication in protocol and interpretation by the seed analyst. For vigor testing to be accepted by the consumer, test results must be standardized among laboratories. This can best be assured by establishing a certification process for seed vigor testing laboratories. Laboratories would be examined to determine that they have appropriate testing equipment. Seed analysts would be required to demonstrate that they were proficient in the conduct of seed vigor tests. Such a certification process provides the seed consumer the confidence that vigor testing provides meaningful and reproducible results. It also provides laboratory control to ensure that the tests are conducted using the exacting standards necessary for standardization of seed vigor test results.

The Next Step

This treatise has identified many of the remaining constraints to routine seed vigor testing. The future of seed vigor testing is indeed bright. Many of the social and technical hurdles are now being addressed. Greater consensus and harmony among seed vigor scientists will surely occur in the future. This vital collaboration will culminate in improved techniques and commonly accepted vigor testing protocols. Already these have resulted in a seed industry that routinely surveys all commercial lots for seed vigor prior to marketing and distribution; evident testimony of the importance of seed vigor to seed quality.

Where do we go from here? While noting the important achievements of the AOSA Vigor Testing Committee in formulating vigor tests for the seed industry, McDonald (1994) concluded with the following observation: “Despite these important achievements, this (seed vigor) information has yet to be routinely provided to the consumer. While development of new and refinement of old vigor tests will be a continuing responsibility, this charge – permitting the seed purchaser the opportunity to read and evaluate vigor test data – remains the future and most challenging role for the Chairs of the AOSA and ISTA Vigor Tests Committees.” To accomplish this objective, the next logical step in the development and use of seed vigor tests should be their incorporation into the Rules for Testing Seeds. Indeed, it is wise and prudent that AOSA and ISTA provide recommendations concerning appropriate vigor test procedures just as they have for germination and purity tests. This process should neither be hasty nor incomplete. All aspects of seed vigor testing should be thoroughly studied and extensive referees of vigor tests conducted prior to incorporation into the “Rules.”

The process, however, should begin and a likely candidate for inclusion into the “Rules” stands at the forefront of the others: the conductivity test. This seed vigor test has been thoroughly studied and correlated with seed vigor. It is widely accepted as a useful seed vigor test and, as a result, is included in both the AOSA and ISTA Seed Vigor Test Handbooks. The test variables have been comprehensively

evaluated (Loeffler, TeKrony, and Egli 1986) and revised test procedures provided. Vigor test referees have consistently demonstrated that conductivity test results are reproducible within and among seed laboratories (Tao, 1980; Reusche, 1987).

Let it be emphasized that incorporation of the conductivity test into the “Rules” does not mean that seed lots must be tested for vigor and that the result should appear on the label. It does mean, however, that when seeds are tested for vigor using the conductivity test that there is a specified procedure that must be followed. This process assures standardization of seed testing, appropriate interpretation of results, and credibility and confidence in the testing protocol. With this recognition will come an increased demand for this additional seed quality information from the consumer.

In conclusion, this text has emphasized the continuing issues that confront seed vigor test standardization. Hopefully, the ISTA and AOSA Vigor Test Committees will address these and other concerns as one single Seed Vigor Testing Handbook emerges. Seed vigor tests provide valuable seed quality information not identified by the standard germination test. It is currently of important value to the seed industry. Subsequent work on the standardization of seed vigor tests will assure that this useful seed quality information also will be available to the seed consumer in the future.

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