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ASSESSMENT OF SEED QUALITY[1]

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The assessment of seed quality continues to attract increasing attention from the seed industry. Farmers believe that seed quality information will enable them to make economic decisions regarding the cost of seeds, earliness of planting, quantity of seeds to plant, and the anticipated uniformity of stand. Seedsmen believe that seed quality information will aid them in monitoring seed quality during the various processing phases of seed production. Seed quality tests might reveal where loss in seed viability occurs during combining, cleaning, drying, storing, bagging, etc. and may pinpoint adverse practices which could subsequently be improved. The accurate assessment of seed quality could have a significant impact on improving seed performance, which would culminate in important economic considerations for the farmer and seedsman alike. This report will attempt to clarify what seed quality is, what constraints are imposed on seed quality testing, how seed quality tests are assessed, how seed quality tests can be standardized, and what the future of seed quality testing may be.

What is seed quality?

The Federal Seed Act dictates that seed quality is determined by 2 principle factors: seed purity and germination. Seed purity tells the seedsman and consumer how much unwanted material is present in the desired pure seed and specifies the nature of each containment. Seed germination is intended to assess how viable the purchased seed is and is defined as, *"the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions"* (1). However, this definition and the general philosophy of germination testing have proven inadequate for assessing potential field emergence for the 2 following reasons:

1. The definition refers to the ability of a seed to produce a normal plant under *favorable* conditions. When seeds are germinated, only optimum test conditions such as substrate, moisture, temperature, light, and adequate testing time are employed to insure that maximum germination percentages are obtained. Unfortunately, optimum conditions are rarely encountered in the field and it is not surprising that field emergence is often less than that predicted by the germination test.

2. The germination test fails to take into account the progressive nature of seed deterioration. A seed is considered germinable when it has fulfilled the criteria established by the definition for the production of a normal plant. Therefore, seeds are classified as either germinable or non-germinable with no distinctions provided for "strong" or "weak" germinable seeds. Such information would be useful since we can forecast that "weak" seeds will soon deteriorate and be classified as non-germinable. Germination testing, therefore, does not provide a complete evaluation of seed lot deterioration or quality.

These inherent weaknesses of the seed germination test have been with us a long time and have resulted in dissatisfaction among seedsmen and farmers. Consequently, interest has been spurred to develop another or supplemental parameter of seed quality. This component is now known as seed vigor and will occupy the major portion of this discussion. Unfortunately, there are many misconceptions concerning seed vigor. This is exemplified by the array of definitions proposed for seed vigor which were recently collated by Heydecker (7). Because of this confusion, it is important that seed vigor be formally defined. The 2 major seed testing organizations, the International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA) have charged their vigor testing committees with proposing a specific definition for seed vigor. In May, 1977, at the Madrid, Spain meetings, the ISTA group proposed the following definition for seed vigor: *"Seed vigor is the sum total of those properties of the seed which determines the potential level of activity and performance of the seed or seed lot during germination and seedling emergence".* Included among the aspects of performance were: 1) biochemical processes and reactions during germination such as enzyme reactions and respiratory activity, 2) rate of

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uniformity of seed germination and seedling growth, 3) rate of uniformity of seedling emergence and growth in the field, and 4) emergence ability of seedlings under unfavorable environmental conditions. Factors which cause changes in the level of seed vigor include the genetic constitution of seed; environment and nutrition of the mother plant; stage of maturity at harvest; seed size, weight, or specific gravity; mechanical integrity; deterioration and aging; and pathogens. This definition is considered an "academic" definition because it discusses, identifies, and describes seed vigor, i.e., it attempts to relay what seed vigor *is*.

In June, 1979, the AOSA Vigor Testing Subcommittee proposed the following definition for seed vigor, *"Seed vigor comprises those seed properties which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions."* This version takes the result of seed vigor and quantifies it in terms of rapid uniform emergence and development of normal seedlings. Thus, this definition focuses on what seed vigor *does* and is, therefore, considered to be an "operational" definition.

Regardless of whether an academic or operational definition is preferred, the emphasis of both definitions is on seed performance under a wide range of environmental conditions. This provides us with specific criteria to evaluate the performance of vigor tests. But, what actually determines seed vigor and what should a vigor test measure? Delouche and Baskin (6) proposed a sequence of events which leads to increasing deterioration, culminating ultimately in loss of germination (Fig. 1). Although this model remains hypothetical and still requires experimental documentation, the authors emphasize that loss of germination is the final consequence of seed deterioration and is preceded by a myriad of changes in biochemical and physiological processes. A closer examination of this deteriorative scheme reveals that those changes which precede loss in germination could serve as vigor tests. Ideally, the ultimate vigor test would be that event which is farthest removed from loss of germinability, e.g., membrane degradation. Many of the vigor tests being considered for standardization are testing one or more of the factors along this path of seed deterioration.

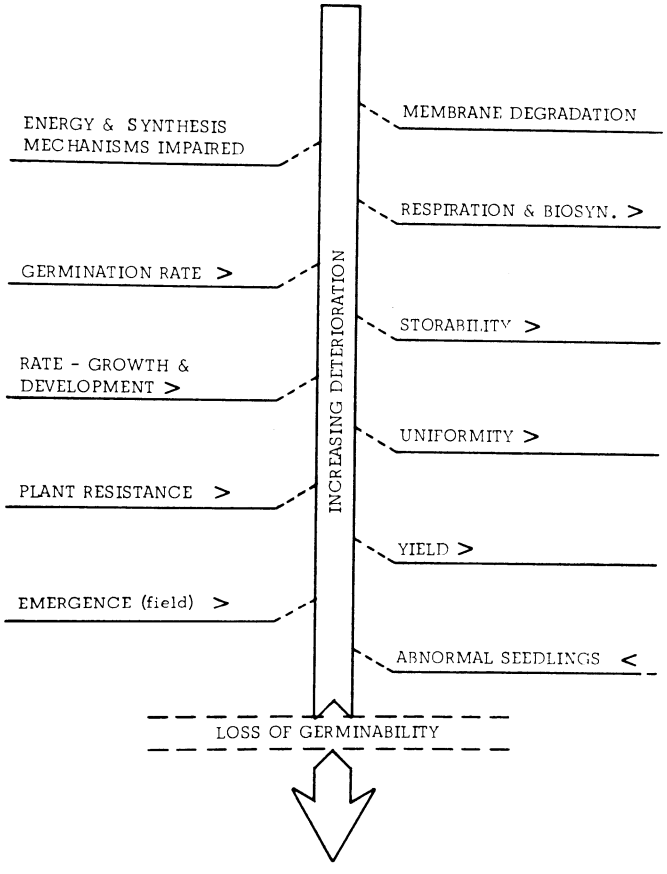

Fig. 1. Probable sequence of changes in seed during deterioration (6).

The constraints imposed on vigor testing.

It would seem from the preceding discussion, that we merely have to develop mechanisms to measure some aspect of seed deterioration prior to loss of germinability. But, it is not that simple. The seed industry has established certain limitations to which each vigor test, in general, must adhere (9). A vigor test must be:

1. Inexpensive. Due to limited budgets in seed testing laboratories, it is important that a vigor test require reasonably priced equipment and supplies.
2. Rapid. Every seed laboratory has periods of peak activity when seed samples arrive for testing simultaneously. During these periods, the addition of another seed quality test in conjunction with the routine germination and purity analyses will place a further burden on the seed analyst. So, it is important that the vigor test be conducted rapidly to keep time spent by the analyst at a minimum. Further, a vigor test which is not rapid will tie up needed germinator space as well as delay the reporting of results to the anxious seedsman.
3. Uncomplicated. A vigor test which requires sophisticated equipment and intricate procedures can become expensive. It may involve detailed training of analysts or may necessitate the hiring of seed analysts with advanced degrees who are capable of understanding and performing the required operations. Where possible, all operations must be simple so that they can be competently conducted in seed laboratories with current size of staff and at a reasonable cost.
4. Objective. For a vigor test to be easily standardized, a quantitative or numerical assessment of seed vigor should be emphasized. Such a system eliminates subjective interpretations by seed analysts, which is the major source of variation in reported results among laboratories. Such a vigor test is a more sensitive measure of seed viability than the germination test, the objective interpretation of vigor results becomes even more critical.
5. Reproducible. The success of any test depends on its reproducibility. If test results cannot be repeated due to intricate procedures, difficulty in interpretations, etc., then a comparison of results between laboratories for the same lot of seed becomes meaningless. Thus, before adopting any vigor test for routine seed testing, we must insure that the results can be reproduced.
6. Correlated with field performance. The preceding definitions of seed vigor emphasize the relationship between seed vigor and anticipated field performance. Recent studies have demonstrated that this association exists (8, 21, 23). Consequently, the ultimate value of any vigor test will be determined by its ability to predict field performance.

Clearly, these specific requisites of a seed vigor test make its selection difficult. Certain vigor tests can adequately quantify seed vigor but they may be unacceptable because they fail to fulfill the requirements listed above (10). Such requirements effectively reduce the number of vigor tests under consideration. However, we must also focus on what we want a vigor test to do. The following questions are pertinent to the development and standardization of a vigor test:

1. Should a vigor test monitor the vigor of a seed lot or of each individual seed comprising the lot? The following hypothetical example is provided to illustrate this question. Two lots of seed are given: lot A possessing 60 seeds at a 100% vigor level and 40 seeds at a 0% vigor level and lot B containing 100 seeds at a 60% vigor level. If a farmer were to plant these lots under adverse environmental conditions, he would obtain 2 different emergence results; lot A clearly would out-perform lot B due to its greater proportion of high quality seeds. The seed analyst may be asked to test these 2 lots of seed for vigor. If the analyst is using a bulk test which evaluates the seeds in groups of 100, he will find that the test will indicate that both lots A and B perform at a 60% vigor rating. On the other hand, a vigor test which evaluates individual seeds will be able to detect the differences in seed quality of lots A and B. Although this example suggests that individual seed evaluation is superior to bulk testing, it must be remembered that bulk testing is more rapid and less expensive than single seed measurements.

2. Should a vigor test be universal for all seeds or designed specifically for certain crops, such as corn or soybeans? Reproducibility of test results is more likely to be achieved when the seed analyst is

required to be familiar with the fewest number of vigor tests possible. This enables the analyst to develop confidence with the tests. Unfortunately, the diversity of seed types, their ability to respond uniquely to adverse conditions, and their distinct genetic constitution may necessitate the use of an array of vigor tests developed for each major crop. This possibility would increase the expense of vigor testing – requiring additional equipment and supplies – and put further temporal and technical demands on the seed analyst. Where possible, development and utilization of a vigor test which can be universally applied to a large number of crops should be emphasized.

3. How should vigor be expressed? In an attempt to make vigor information useful, various investigators have proposed different methods of expressing seed vigor results. One possibility is to use categories such as high, medium, and low. Although these categories have meaning to the seedsman and farmer, they do pose certain difficulties. A categorical system requires cut-off lines to delineate groupings. If it is established that 70% and above is the cut-off point for high vigor seeds, a seedsman possessing a seed lot with a value of 69% may request that his seed be retested in the hope of increasing its rating and subsequent commercial value. The problems of this system become more manifest if tolerance values and test variations are also considered. Other researchers suggest that percentage values are useful. However, this expression is closely related to germination and it is surprising how often germination and vigor concepts become intertwined and confused. Finally, the very nature of the seed vigor test may dictate how vigor test results are reported. For example, the conductivity test expresses results as μmhos/g seed and the seedling growth rate test is expressed as mg dry weight/germinated seedling. These evaluations have little meaning to the seedsman and farmer and will require thorough explanations before they can be adequately interpreted. Whatever system of reporting results is ultimately selected for a vigor test, it cannot be overemphasized that accompanying explanations of the data should be provided.

4. Will one vigor test provide all the information we need or should we design a battery of vigor tests to provide more exact information? Through continued research, we are gaining greater confidence regarding vigor test capability. We know which facets of seed quality each vigor test measures. As a result, it has been suggested that vigor tests be used to complement each other and enable a better assessment of seed viability. However, there is a point of diminishing return where the additional information gained becomes uneconomic. I hope that a single test will prove to be successful for seed testing laboratories as we continue to develop and refine vigor tests.

5. How will dormant and fungicide-treated seeds be evaluated? Some crops are dormant at the time of seed testing and rapidly lose this dormancy by the time of planting. However, since many vigor tests rely upon some quantitative assessment of seedling growth to monitor seed vigor, should dormant seeds be considered low in vigor? Similarly, fungicide-treated seeds often perform better under field conditions than nontreated seeds. Although man has artificially altered the performance capability of these seeds, should seeds be vigor tested in the treated or nontreated condition? These questions must still be addressed when results of vigor tests are interpreted.

How is seed vigor measured?

The constraints imposed on vigor testing have clearly limited the types of vigor tests which are under consideration. Despite these restrictions, however, several vigor tests have been developed and outlined by the AOSA Vigor Testing Subcommittee in *"The Progress Report on the Seed Vigor Testing Handbook"* (22). It is beyond the scope of this report to detail these procedures. However, it is important to list the vigor tests and briefly mention and describe the merits of each test for seed quality assessment.

1. Accelerated Aging test. This test embodies many of the important traits characteristic of a vigor test. Initially proposed as a method to evaluate seed storability, the accelerated aging test subjects unimbibed seeds to conditions of high temperature (41°C) and relative humidity ($\sim$100%) for short periods (3-4 days). The seeds are then removed from the imposed stress conditions and germinated under the optimum conditions specified by the "Rules." This test possesses the following important requirements of a seed vigor test: rapid, inexpensive, simple, universal for all seeds, capability for individual seed evaluation, and requires no additional training for correct seedling evaluation. Although these assets make this test appealing, reproducibility of results within and among seed laboratories has not been achieved. This variation is attributed to many factors. Recent results suggest that a modification of the accelerated aging chamber would be beneficial (2, 14). Another study has shown that differences in initial seed moisture should be considered when interpreting this test (12). As these factors are standardized, the accelerated aging test may become one of our most reliable vigor tests.

2. Cold test. The cold test is one of the oldest methods of stressing seeds and is most often employed for evaluations of seed vigor in corn. Seeds are placed in soil or paper towels lined with soil and kept in the cold for a specified period. During this period, stress from imbibition, cold temperature, and microorganisms occurs. Following the cold treatment, the seeds are removed and placed under favorable growth conditions as specified by the "Rules." The greatest difficulty with the cold test is the inability to standardize field soil (5). Soils differ in moisture, pH, particle composition, pathogen levels, etc., and these parameters contribute to divergent results. Vermiculite, a more easily standardized medium, has recently been proposed as a possible solution to the inherent variability of soil conditions. However, others maintain that a cold test requires field soil to be successful. In spite of these difficulties, seed vigor rankings by the cold test remain consistent within laboratories, which lend support to the premise that this is a most useful "in-house" seed vigor test.

3. Conductivity test. Low vigor seeds have been shown to possess poor membrane integrity as a result of mechanical injury and storage deterioration. When seeds are imbibed, cells having poor membrane structure release cytoplasmic solutes into the imbibing medium. Those solutes with electrolytic properties carry an electrical charge which is detected by a conductivity meter. Measurement of the conductivity of leachates from seeds is a rapid, precise, inexpensive, and simple procedure. However, initial seed moisture (17) and seed size (18) can affect the rate of solute leakage and may require further standardization efforts. Additionally, treatment of seeds with fungicides may influence conductivity measurements, necessitating their removal before determinations are made. The conductivity test also evaluates the leachate of bulk samples rather than individual seeds.

4. Cool germination test. Unlike the cold test, the cool germination test is conducted under standard laboratory conditions at low temperatures (18°C) and does not rely upon the activity of microorganisms to stress the germinated seeds. It has been demonstrated that low vigor seeds from a warm season crop, such as cotton, will culminate in decreased growth rate and germination under these conditions. The major advantage of this test is that it is conducted according to the routine procedures for a germination test and the standard criteria for normal seedlings as specified by the "Rules." Its principal disadvantage is that the test is limited to cotton.

5. Seedling growth rate test. Vigorous seeds are able to efficiently synthesize new materials, rapidly transfer these new products to the emerging embryonic axis, resulting in an increased dry weight accumulation. The seedling growth rate test is based on this concept and vigor results are consequently expressed as mg dry weight/germinable seedlings. This test is generally conducted according to the standards for the routine germination test. After germination evaluations are made, the growing segments of the embryo from normal seedlings are excised from the storage organs (cotyledons or endosperm) to minimize seed size differences, placed into beakers, and dried at 80°C for 24 hours. Following the drying period, dry weight is determined to ascertain the dry weight increase. Since seedling growth rate is correlated with vegetative development in the field (4, 16), this test offers substantial promise. However, certain standardization factors still need to be addressed. Small differences in moisture and light intensity can have significant effects on the rate of seedling growth. This test may also require standardization for specific cultivars since rate of seedling growth can be under genetic control (3).

6. Seedling vigor classification test. This vigor test is an expansion of the routine germination test, requiring the seed analyst to further classify "normal" seedlings into "strong" and "weak" categories. Since the test requires no additional equipment and employs concepts and terms familiar to seed analysts, this test becomes particularly attractive. Despite these immense advantages, however, this vigor test is faced with one serious standardization difficulty. The correct evaluation of germination by seed analysts is difficult to obtain because it is subjectively assessed. For this reason, the AOSA and SCST maintain active referee programs that identify laboratories with interpretive germination difficulties. To further separate "normal" seedlings into 2 additional categories is a subtle task and perhaps beyond the reach of the average seed analyst.

7. Tetrazolium (TZ) test. The TZ test is one of our most valuable seed analysis tools. This test relies upon the action of the TZ molecule to react with hydrogen atoms released as a result of the activity of dehydrogenase enzymes to form a water insoluble red pigment

called formazan which identifies living tissues. A trained seed analyst evaluates the seeds for staining pattern, color intensity, and subjectively places the seeds into prescribed vigor categories which range from strong to weak. This vigor test correlates well with seed vigor in the hands of a trained analyst but is subject to certain standardization difficulties. Foremost among these is the ability of a technician to ascertain whether a seed is vigorous. Such evaluations require considerable training in TZ staining and embryo morphology. The TZ test also fails to detect seed treatment phytotoxicity, heat injuries incurred from artificial drying, and fails to reveal seed dormancy (9). However, an extensive educational program that emphasizes interpretation of stained seeds could result in consistent and reliable TZ evaluations.

How can vigor tests be standardized?

Clearly, the number of vigor tests being considered for adoption into routine seed testing programs is indicative of the amount of standardization work remaining. It is not possible that all the vigor tests described here can be used effectively in 1 seed testing program. Therefore, there must be a mechanism by which an analysis of vigor test capability can be attained. Such a system would allow us to discard less useful tests and concentrate on those tests displaying the most promise. To undertake such an evaluation, the AOSA Vigor Test Subcommittee established a vigor test "referee" program initiated in 1977. During 1977 and 1978, a questionnaire was forwarded to over 150 AOSA and SCST laboratories requesting their participation in the "referee" program as well as determining the need for standardization of vigor tests. The following responses were obtained to 2 questions:

1. Is your laboratory currently evaluating seeds according to vigor?

	1977	1978
Yes	38	54
No	35	46

2. What seeds are generally vigor tested by your laboratory?

	1977	1978
Corn	27	34
Soybeans	23	28
Sorghum	10	4
Cotton	8	9
Beans	8	8
Peanuts	8	3
Lettuce	5	5

About half of the laboratories that responded were conducting seed vigor tests and corn and soybeans were the most popular crops evaluated. Thus, the AOSA vigor test "referee" program included corn and soybeans in each year's study. Lettuce, cotton, and wheat were evaluated in 1978 and cotton was included with corn and soybeans in the 1979 "referee." Written guidelines for conducting each vigor test accompanied the seed samples to insure that test procedures were identical among laboratories. The complete results and discussion of these data have been presented elsewhere (11, 13, 15, 19, 20) and only a synopsis of the results will be provided here.

Three criteria were analyzed in the "referee." These included:

a) Which vigor test(s) can accurately separate seeds into appropriate vigor categories?
b) Can the testing laboratories reproduce their results?
c) Can the testing laboratories reproduce results of other laboratories?

Following receipt of the data, a statistical analysis was conducted to address these questions. In the 1979 AOSA vigor test "referee" for soybeans, the results showed that there was no difference in the germinations of the 3 samples tested but that the 3 vigor tests studied predicted a difference in field emergence (Table 1). Samples 2 + 6 were the highest seed quality, samples 1 + 5 were intermediate, and samples 3 + 4 were the lowest quality. It should be emphasized that these are commercial quality (germination above 80%) soybeans and that no indications of potential field performance could be determined from the germination results. However, when these same soybean lots were planted under field conditions, an obvious difference in rate and final emergence was demonstrated (Fig. 2). These data clearly illustrate the importance and usefulness of seed vigor information to the farmer and the seedsman.

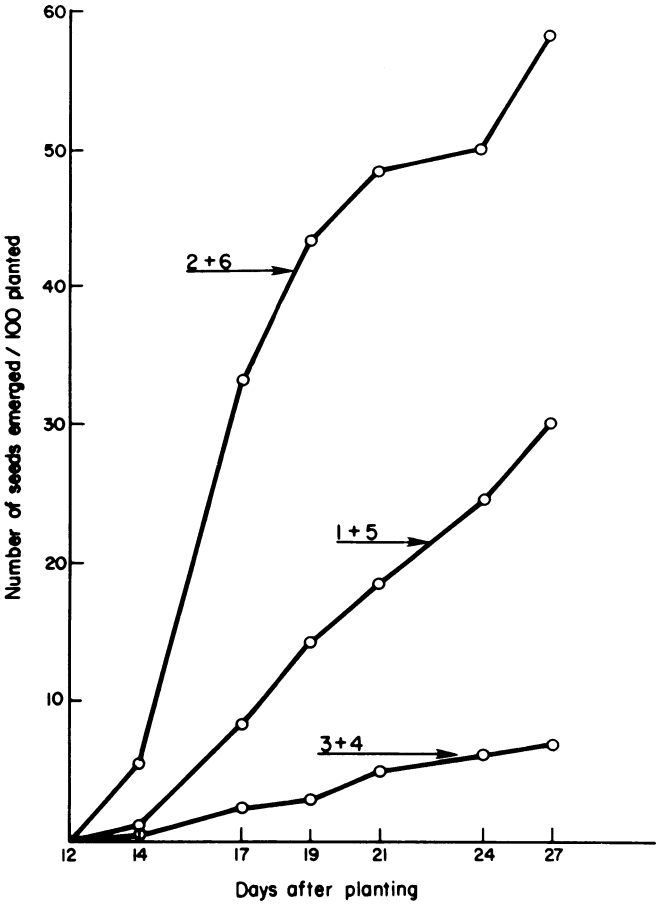

Fig. 2. Rate of emergence of soybeans of 3 vigor levels as used in the 1979 AOSA vigor test "referee" planted on April 27 (20).

The value of seed vigor tests for assessing seed quality has, of course, been recognized for a long time. Still, relatively little effort has been directed at standardizing these tests and determining which tests offer the greatest accuracy in measuring seed quality. Standardization of test procedures and interpretation of results is important because it enables the seed industry to provide useful information to the consumer in a manner which is verifiable. Standardization would eliminate the confusion presently associated with vigor advertising because the results would be reproducible. The AOSA vigor test "referee" program has been designed to address some of these difficult issues. The 1979 "referee" for soybeans again showed that no significant difference in performance existed for the 3 seed lots as detected by the germination test but that the accelerated aging, cold test, and conductivity tests were able to provide a better indication of final field emergence (Table 1). Further, no statistical difference in results was reported from laboratories using the standard germination, accelerated aging, and conductivity tests (Table 2). Such results indicate that these tests and test procedures are nearing standardization for soybeans and offer future promise for the routine use of these tests in seed quality analysis. However, a significant difference in results for the cold test among laboratories was noted. Since the cold test relies on microorganism activity for optimum performance and since soils differ in microorganism content

Table 1. Mean results of 3 soybean seed lots for standard germination, field emergence, accelerated aging, cold test and conductivity during the 1979 AOSA vigor test "referee" (20).

Sample no.	Standard germination (%)	Field emergence (%)	Accelerated aging (%)	Cold test (%)	Conductivity (μmhos/g seed)
1 + 5	89	31	30	24	89.6
2 + 6	91	58	86	62	76.8
3 + 4	95	8	0	3	111.6

Table 2. Number of laboratories participating and the statistical analysis of the results of 3 soybean seed lots for standard germination, accelerated aging, cold test, and conductivity during the 1979 AOSA vigor test "referee" (20).

No. of laboratories	Test	Statistical analysis: Among laboratories	Statistical analysis: Seed lots
22	Standard germination	NS	NS
15	Accelerated aging	NS	**
14	Cold test	**	**
4	Conductivity	NS	**

NS = Not Significant.

** = Significant difference at 1% level.

from region to region, this variation was not surprising. Further standardization efforts are needed for the cold test in order that this valuable vigor test can be used to make seed quality comparisons among laboratories. It should be emphasized that such results do not diminish the use of the cold test as an "in-house" tool for distinguishing seed quality among seed lots.

The AOSA "referee" program has proven to be an effective mechanism to evaluate vigor test performance as well as focusing on difficulties which routine seed testing laboratories encounter while conducting vigor tests. As these problem areas are resolved through the "referee" program, solutions are developed which permit further standardization and use of these valuable instruments for assessment of seed quality.

The future of seed vigor testing.

Vigor testing is imminent. We are only beginning to achieve a degree of confidence in the reliability and capability of certain tests. Further research and testing are still required before vigor testing becomes a routine phase of seed testing. Clearly, any advertising of seed vigor is premature at this point. Unfortunately, due to the demand by seedsmen and farmers for this increased seed quality information, vigor results have already begun to appear on seed labels. The attitude of certain seedsmen to be the first to advertise seed vigor or, at the very least, to meet the demands by their competitors, is certain to result in seed enforcement. It is hoped that seed enforcement officials will not overreact to this competitive response and penalize the careful seedsman who is trying to produce and provide the best quality seed available. However, should legislation be enacted, the law should possess the following salient features to allow further standardization of vigor tests and still enable the responsible seedsman the opportunity to market his seed according to vigor:

1. Advertising of seed vigor should be strictly voluntary. Many seedsmen already test their seed for vigor. Should a seedsman find that his seed has an acceptable germination but a low vigor rating, he will most likely decide not to provide this additional quality information since it will probably detract from the value of his seed. This insures that only high quality seed will be marketed according to vigor; a kind of seed that seldom presents enforcement officials with legal problems. At the same time, this provision allows the seedsman to chose what he wants the consumer to know about his seed and provides the farmer with the vigor information he desires.

2. If seed vigor is advertised, test methods should be divulged and test results verified by an independent laboratory. Because of the diversity of vigor tests and the difficulties in standardization, test results should be confirmed for consumer protection. This provision eliminates "secret" vigor tests, anomalous descriptions such as "vigor verified", and also insures that seedsmen conduct the test responsibly. It protects the cautious and reliable seedsman against the competitor who is too willing to prematurely accept vigor test results for advertising purposes.

3. Vigor tests should be repeatable or a "stop sale" order can be issued. Vigor test results may not be reproducible because the vigor test requires further standardization or the test results are not as claimed. If the former situation applies, the vigor test requires additional research and should not be used in commercial advertising until it is refined; if the latter, the vigor information should be questioned and the seed should not be sold until the vigor advertising is removed. Such an enforcement procedure insures that only responsible seed vigor advertising occurs in the marketplace.

There is little doubt that the seedsman and seed consumer want more information about the quality of seed than the germination test alone provides. The infancy of seed vigor testing and the many standardization problems still needing to be resolved give little credence to advertised seed vigor claims until specific vigor tests are approved by unbiased seed testing organization such as the AOSA and ISTA. There will continue to be an upsurge in interest, use, and advertising of seed vigor. In the meantime, the seedsman must insure that germination and purity information meet the quality standards required for the crop planted. As vigor tests are developed for commercial use, they will provide an additional supplement which will allow the seed purchaser to make an accurate assessment of seed quality.

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