THE STAND AND plant producing potential of cottonseed is most commonly evaluated by a germination test. This test is the most widely used and recognized means for measuring the physiological quality of seed. Procedures for determining the percentage of germination have been perfected and standardized so that different laboratories obtain remarkable uniform results when testing seed from the same lot.

The standard germination test appears to serve admirably the needs of the seed analyst and seed control official. But does it also serve the needs of the seedsman and farmer? This question has been asked so many times in so many different ways in the past 10 years that it has become commonplace and even rather tiresome. Yet, any answer is still likely to stir the emotions and rhetoric of those that take the yes or no positions and even those who do not take any position.

Our answer to the question is that the germination test does not adequately serve the needs of the seedsman who produces, processes and sells the seed or the farmer who plants it. And, the inadequacy gap increases with each advance in mechanization, input level, and cost of production. The farmer, particularly, needs some assurance that seed represented to be of high quality are indeed capable of producing a rapidly
One replicate (50 seed) from a standard germination test of cottonseed six days after planting at 86°F. Dead seed and abnormal seedlings are at extreme right. Note variability in length of normal seedlings.

Low temperature germination test results of two lots of cottonseed six days after planting. Top, high vigor seed. Bottom, medium vigor seed. Both lots germinated 85% in the standard test.
emerging, uniform stand of healthy, vigorous plants. Although the farmer is a realist—he is too close to nature to be otherwise—and does not expect miracles from seed or any other agricultural input, he does suspect that something is just not right when he obtains a poor stand of variable size plants from 80% germination cottonseed, while his neighbor across the way gets a good stand from seed of the same germination but of a different lot. His suspicions are even more aroused when seed with similar germ but from different lots perform completely different in his own field.

**Germination Test Deficiencies**—The inadequacies of the standard germination test as a measure of the physiological quality of seed stem from two sources: the philosophy of germination testing and the nature of seed deterioration. The philosophy of germination testing is simply this: that the test conditions and the test period should be such that maximum germination percentages are obtained. To some extent the ideal of maximal results is tempered by the concept of “normal” and “abnormal” seedlings. Practically, however, interpretation of germination test results only eliminates the dead, badly diseased, and the totally and irrevocably lame from the germination percentage. The weak, the semi-lame and the strong count the same in computing test results.

A germination test of cottonseed is made by planting four replicates of 100 seed each of moist paper towels. The towels are folded or formed into rolls and incubated at an alternating temperature of 68° to 86°F. for 12 days, or at a constant temperature of 86°F. for 8 days. One or more preliminary evaluations are made before the final interpretation at the end of the test period. Seed that produce seedlings which have at least one cotyledon (seed leaf), an unblemished stem, and a primary root, or if the primary root is missing, secondary roots, are counted as germinated.

Most farmers will easily recognize that the test conditions of ideal, controlled, moisture and temperature seldom prevail in the seed bed. Thus, the germination test is an inadequate measure of the stand and plant producing potential of seed because the conditions under which it is made

<table>
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<tr>
<th>Lot No.</th>
<th>Laboratory Germination (%)</th>
<th>Field emergence (%)</th>
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<th>35 days*</th>
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<td>B-14</td>
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*Percentage of plants surviving after 35 days.
Soil cold tests results of three lots of cottonseed. Germination percentage of all three was 80 to 85%. H, L, and M refer to high, medium and low vigor.

are totally free of the stresses normally encountered in the field, and no distinction is made between seed that germinate rapidly and produce vigorous completely “normal” seedlings, and those that germinate slowly and produce weak seedlings with parts detached.

With emphasis on germination—as defined in the rules for testing seed—all these years, attention has been focussed on the final and most disastrous consequence of seed deterioration to the complete neglect of its “lesser” effects. The performance potential of a non-germinable seed is 0%, but it does not follow that the potential of every germinable seed is 100%! Rather, it is somewhere in the range 1% to 100%. The less deteriorated the seed, the further up the scale is its performance potential, and the higher is its vigor.

It has been well established that many changes detrimental to performance occur in seed before they become incapable of germination: rate of germination and seedling growth decreases, the range of conditions over which the seed will germinate and emerge narrows about the optimum and susceptibility to microorganisms or any other stress increases. Therefore another reason why the germination test is an inadequate measure of the physiological quality of seed is that it is not a very sensitive measure of quality.

The deficiencies of the germination test are strikingly evident from the data in Table 1 comparing laboratory and field performance of several lots of cottonseed. The field tests were conducted in the same plot at the same time.

Although we have treated the germination test rather harshly, we do not advocate abandonment of it. It can be effectively used provided its limitations are recognized. Germination percentage, probably, still is the best quality index for labeling and seed control purposes. Test procedures are highly standardized and results are reasonably uniform among laboratories—essential characteristics for the orderly movement of seed in marketing channels. The germination test also effectively discriminates among seed lots that differ widely in germination percentage. A seed lot that germinates 65% will not—on the average—perform as well as a lot that germinates 75%, which in turn cannot be expected to give the performance of a lot that germinates 90%. The germination test really “breaks down” in its failure to differentiate among quality levels of lots in the same germination range, say 80 to 85%? Since most cotton seed lots marketed germinate in this range, the importance of the deficiency is apparent.
Deficiencies of the germination test have been discussed, and the question now arises: Are there other tests that can be used to obtain more meaningful information on the stand and plant producing potential of seed? Indeed, there are other tests that are more effective than the germination test and most of them can be classified as vigor tests.

The basic objective of testing seed for vigor is to differentiate among quality levels of seed lots with essentially the same germination percentage. This is accomplished by evaluating the performance of the seed under conditions that are less than optimal. One or more stresses are applied that permit survival of only vigorous seed.

The effect of stress on seed performance and its relation to quality of vigor is difficult to explain except by analogy. Assume that we randomly select 100 adult males from a population and set up two tests for them. The first test evaluates their ability to walk one mile without resting, while the second evaluates their ability to run the same distance without resting. All the entrees except possibly 1 or 2 will complete the walk test although the time required will vary widely. The number of entrees who complete the

Seedling growth rate vigor test results from 3 lots of cottonseed after 4 days at 77° F. Top, high vigor; center, medium vigor; bottom, low vigor. Lab germination of all 3 lots was 80 to 84%.
run test, however, will be quite low, probably less than 30%. Only the vigorous and physically fit can make it. The germination test may be likened to walking and vigor tests to running.

Vigor Tests—Development of vigor tests is one of the most intensely cultivated areas of seed research. We can expect considerable advancements in the techniques and effectiveness of vigor tests in the next few years. There are, however, at least three types of vigor tests that have been developed sufficiently for use by cottonseed producers, processors and farmers in identifying high quality seed lots. These are the “cold tests”, the rate of germination and/or seedling growth tests, and the tetrazolium test.

Cold Tests—Two variations of “cold tests” are available for vigor testing of cottonseed: “the low temperature germination test” and the “soil cold test”. The low temperature germination test is made in the same manner as the standard germination test (seed planted in rolled paper towels) except that a suboptimal temperature is used—usually 65°F. Test results are interpreted in terms of the percentage of seed that germinate and produce normal seedlings at least 1½ inches long in 7 days. Vigorous seed give high test results (70% plus), while aged, deteriorated seed of low vigor give low percentages. The low temperature germination test is easy to make and within the capability of any laboratory equipped to test seed.

The soil cold test is a vigor test that simulates the three most common stresses cottonseed are subjected to in the seed bed: low temperature, wet poorly aerated conditions, and a population of microorganisms. Test procedures are as follows:

Top soil is taken from a cotton field, screened, mixed with builder's sand to a good consistency, and stored in covered steel drums. About 1 inch of the mixture is placed in the bottom of a plastic crisper box (app. 7” wide, 10” long and 4” deep), 100 seed are planted, and covered with another inch of the soil-sand mixture. Moisture content of the mixture is then adjusted to 60% of saturation by adding a predetermined amount of water. Two are more boxes (100 seed each) are used for each test. The boxes are covered and incubated at 54°F. for 3 days. After the incubation period the tests are transferred to a warm temperature (80 to 86°F.) for emergence. After about 4 to 5 days the tests are ready for evaluation.

Results are expressed as the percentage of normal seedlings that emerge. Since soil cold tests vary with the type of soil used, each laboratory has too adjust the severity of the test by increasing or decreasing the low temperature incubation period. The procedures described above are those used in our laboratory. We consider that a cottonseed lot is low in vigor if cold test emergence is below 60%. Emergence percentages of 80% plus are obtained from the very highest quality seed. Since fungicide treated seed invariably preform better in the soil cold test than untreated seed of the same lot, comparisons are valid only when all lots tested are treated or all are untreated.

Rate of Germination and Seedling Growth—The vigor of seed is manifested in the rate (speed) of germination and seedling growth. Vigorous seed germinate rapidly and the seedlings produced grow rapidly. Both of these characteristics are used to assay vigor. Speed of germination is determined by planting the seed in the same manner as for the standard germination test including the same favorable temperature. The only difference is that the tests are evaluated after 3 days at a germination tem-
Cottonseed embryos stained with tetrazolium. Red stained (grey) areas are live, healthy tissue. White areas are dead tissue. Only the seed at the extreme right, bottom row, is capable of germination.

Temperature of 86°F. or after 4 days at the alternating 68° to 86°F temperature. Normal seedlings that attain a minimum size of 1 1/2 inches are counted. The higher the percentage germination in the specified periods the higher is seed vigor. Top quality seed should “germinate” at least 70% in the 3 or 4 day periods.

Rate of seedling growth is determined by planting the seed with root ends oriented in the same direction in a row on moist paper towels (about 15 seed per towel). The towels are rolled and placed in an inclined position in the germinator, so that the seedlings produced will grow straight and in the same direction. After 3 to 5 days, depending on whether an 86° or 77°F. temperature is used, the average seedling length is determined by measurement. At least 50 seed should be planted for each test. Vigor is related to the length the seedlings attain during the specific period. The greater the average length of the seedlings the higher the vigor of the seed.

A major limitation of the speed of germination and seedling growth tests is that they are not very effective in evaluating freshly harvested cottonseed. Cottonseed in many lots retain a sort of residual dormancy for 2 to 3 months after harvest that causes them to germinate slowly.

Tetrazolium Test—The embryo or “meat” of a cottonseed is the essential living part. As the seed deteriorates, various portions of the seed weaken and die. Depending on the site and extent of dead tissue the seed may lose its capacity to germinate. A considerable portion of the cotyledons or seed leaves can die without affecting germinability although vigor is much reduced. On the other hand, a very small dead area on the embryonic root renders the seed non-germinable even though the rest of the embryo is live and apparently healthy.
Dead and live tissue cannot be distinguished without the use of some stain. The tetrazolium test is widely used to differentiate between dead and live tissue. This test involves treatment of the embryo (the hull has to be removed) with a dilute solution of tetrazolium chloride, which is soluble and colorless in water. Live tissue has the capacity to reduce the chemical to a red stain, while dead tissue does not. Thus, live tissue stains red while dead tissue retains a natural color (yellow-white). Careful analysis of the pattern of living and dead tissue permits classification of individual seeds as to both germinability and vigor.

The tetrazolium test does not require much equipment but it is tedious and considerable experience is necessary to properly interpret the test results. Nevertheless, it is probably the most effective method of evaluating vigor of cottonseed or any other kind of seed.

Uses of Vigor Tests—Vigor test results provide much more meaningful information on seed quality than the regular germination test. While we do not advocate that vigor tests completely replace the germination test, we do believe they should be applied as a supplemental test.

Seed producers can use vigor test results as an additional basis for selection of high quality seed for processing and marketing. Wholesalers and retailers can use vigor test results to select only high quality seed for their stocks and as a basis for adjusting price to quality. The cotton farmer in addition to being the ultimate beneficiary of improved quality control at the producer-seller level can use vigor test results to check on the quality of the seed he buys or saves and to adjust planting rate and time.