Attaining seed and seedling vigor in cotton is a goal of both researchers and producers. By separating and defining components of seed and seedling vigor, progress can be achieved. Seed vigor should be distinguished from seed viability and defined in terms of low degree of seed deterioration. Varying levels of resistance to seed deterioration have been achieved using different approaches. Three components are proposed for defining seedling vigor: well-developed seedling roots, rapid true-leaf differentiation, and low incidence of seedling disease. Approaches for making improvement in each of these components are discussed. Attaining seed and seedling vigor, and subsequently vigorous stands of cotton, requires an integrated approach and improvement in each component.

Cotton (Gossypium hirsutum L.) plants are vulnerable to various stresses during the seed and seedling stages. Cathey (1985) provided evidence of the importance of these growth stages by finding a significant, positive correlation between yield and heat unit accumulation only during the seedling stage at Stoneville, MS, for a two-week period in May. Similarly, work in California indicated that cotton yields increased as more heat units, up to a critical point, were accumulated during the first five days after planting (Kerby et al., 1989). Both studies indicated that warm temperatures after planting and, by inference, vigorous seedling emergence and development are critical for high yields and efficient cotton production. In contrast, Pettigrew and Meredith (2009) found that the negative association between poor seed quality and lint yield could be mostly avoided by adjusting the seeding rate. However, they also stated that “common sense dictates that producers should avoid utilizing poorer quality seed or planting early into cool and wet conditions on fields with a past history of seedling disease or drainage problems.”

At least four factors contribute to low seed and seedling vigor in cotton: 1) cotton originated in tropical/subtropical regions as a perennial shrub. Consequently, high seed and seedling vigor was not essential for survival, and little natural selection for vigor occurred. 2) The indeterminate growth habit of cotton negatively influences seed and seedling vigor. Due to its indeterminate fruiting, bolls on a plant develop sequentially. Consequently, nutritional and environmental stress on seed in different bolls varies. Variation in boll development leads to bolls opening over an extended period, which causes differential exposure to seed deterioration after boll opening. 3) Cotton is frequently planted early to extend or modify the potential fruiting period. Early planting in many areas increases the probability that harsh conditions will be encountered in the seed and seedling stages. 4) Seed quality in cotton has received less attention than seed quality in grain and cereal crops, partially because the primary value of cotton is not derived from seed production but from its fiber. Consequently, fiber quality often has been given more emphasis than seed quality.

The term “seed and seedling vigor” is frequently used by producers, seed companies, extension workers, and researchers, but the term is seldom defined. Before seed and seedling vigor can be improved, it must be defined and characterized. Obviously, seed characteristics are intrinsically related to seedling characteristics. However, seed vigor and seedling vigor are addressed separately here, and improvement strategies for each are discussed.

SEED VIGOR

Seed Vigor Defined. Niles (1967) suggested five criteria for describing high cottonseed quality: 1) sound seed coats and free of damage; 2) no internal infection by seedborne pathogens; 3) processed to remove immature seed, excess lint, foreign seed, and extraneous matter; 4) uniformity of size; and
5) high germinability and emergence over a range of environmental conditions. If the final criterion is met, the other four become relatively unimportant. However, accurate assessment of this criterion is made difficult by the “range of environmental conditions” qualification. Seed that germinate and emerge only under optimum conditions do not possess high vigor. Vigorous seed are not only able to germinate and emerge in optimum conditions but are able to tolerate harsh conditions.

The ability to germinate relative to a range of environmental conditions can be described as the difference between viable and vigorous seed. Viable seed possess live tissue and germinate at optimum conditions. Viability is easily evaluated by a standard, warm temperature germination test that provides an indication of field emergence under favorable conditions. But standard germination test results are a poor indicator of emergence if suboptimum conditions are experienced and only vigorous seed emerge. Vigorous seed are those with little dead tissue and the ability to germinate in poor conditions. Accurate assessment of seed vigor is more evasive than measurement of seed viability, partially because poor conditions encompass a much wider range of environments than those identified as optimum conditions.

The association between viability and vigor is well described by the seed quality (or germination) curve (Fig. 1) developed by Bird and Reyes (1967) and confirmed by Bourland and Ibrahim (1982). Until cottonseed have been conditioned for germination by exposure to heat and moisture (factors of deterioration), they will not produce maximum germination even under favorable conditions. Maximum germination is attained by conditioning the seed with incremental exposure to heat and moisture, which typically occurs after boll opening and prior to harvest, but can occur during processing and storage or even after the seed are planted. Once maximum germination is attained, additional exposure to heat and moisture causes germination to decline. If a seed lot having standard germination of 80% is entering the conditioned stage (left of the curve apex), the seed would be vigorous. A second seed lot can also have 80% germination but might be entering the deteriorated stage (right of the curve apex) and, thus, have low vigor. Therefore, standard germination tests can provide estimates of both viability and vigor if the conditioning status of the seed can be established.

In their tests, Bird and Reyes (1967) found that the proportion of seed coats with visible mold growth were similar for both unconditioned and conditioned seed but increased sharply as seed deteriorated. Therefore, the seed quality of the two seed lots, both with 80% germination, could be differentiated by germination percentage in combination with the amount of mold growth. Also, they found that as seed deteriorated, seedlings developed more abnormal roots, were shorter, emerged more slowly, and produced lower stands. Based upon the seed quality curve, a working definition of seed vigor can be expressed in terms of low degree of deterioration. Seed can be vigorous either because they have little exposure to deterioration conditions or because they have been able to resist deterioration, that is, deteriorate slowly and maintain germinability.

Evaluating Seed Vigor. Methods of determining seed quality include the frequently used standard germination test that gives a measure of seed viability. Cottonseed are tested at either alternating 20 to 30 °C or at a constant 30 °C (AOSA, 1993; Hake et al., 1990). The cool germination test developed by Bird and Reyes (1967) provides an indication of vigor, but at 18 °C slight variations in temperature can cause large differences in germination. Cool test results on one lot of seed can vary between laboratories or between runs at the same laboratory. If optimum field conditions are experienced, cool test germination percentage can underestimate field emergence. The cool-warm vigor index test adds germination percentages from cool and standard tests together (Gregory et al., 1986). This sum or index then will fall within various quality groups that have prescribed recommendations on use of the seed. Results from the cool-warm vigor test provide a
A good indication of seedling emergence rate and final stand survival (Kerby et al., 1989; Metzer, 1987).

Stress tests employ the determination of seed viability after the seed have been exposed to adverse conditions. The most common of these techniques is accelerated aging (Delouche and Baskin, 1973; Presley, 1958). Cottonseed are aged for 72 h in a chamber where warm, moist conditions (42 °C at nearly 100% relative humidity) are maintained. Difficulties associated with this test are primarily associated with the time required to age the seed, which delays results, reduces the testing capacity of a laboratory, and invites error due to microorganisms and mechanical problems.

Other stress methods include the methanol stress test and the hot water stress test. Musgrave et al. (1980) found that pre-germination treatment of soybean (Glycine max L.) seed with methanol-water solutions mimicked the effects of accelerated aging. Hernandez et al. (1988) demonstrated that the methanol stress technique also was effective for altering viability and vigor of cottonseed.

The hot water stress technique combines the two physical factors of deterioration, heat and moisture, into one medium (hot water). Bourland et al. (1988) found that hot water stress mimicked accelerated aging. Acid-delinted cottonseed were immersed in hot water (for specified times and temperatures), dried for 24 h, and then tested for germinability. As water temperature increased from 50 °C to 90 °C, time required to kill 50% of the seed decreased from 283 min. to less than 1 min. (Fig. 2). Based on these findings, cottonseed are immersed into hot water (65 °C) for specified periods of time then tested for viability at 30 °C using conventional methods. A water temperature of 65 °C was considered optimum for deteriorating seed. Treatment at cooler water temperatures required excessively long treatment periods that would limit the quantity of seed being treated within a given time period and sometimes would result in initiation of the germination process. At higher temperatures, maintenance of a constant temperature throughout the treatment period and uniformly on all seed was difficult, and variation in resistance to deterioration might be masked or altered by minor differences in seed size, seed coat configuration, among others. Deterioration using the hot water technique provided more consistent results than deterioration by accelerated aging (Furbeck et al., 1989). Furbeck et al. (1993a) confirmed that germinability after hot water treatment can be used to predict resistance to field weathering. The hot water technique could be used as a method for evaluating seed quality, which would be analogous to the cool-warm vigor index. Five minutes of hot water treatment conditions any unconditioned seed to germinate so that an accurate measure of viability can be attained. Germination after 35 to 45 min. provides an indication of vigor. The test is referred to as “rapid” because final germination is obtained in approximately 36 h at 30 °C.

Figure 2. Time required to kill 50% of cotton seed at hot water temperatures ranging from 50 to 90 °C (reprinted with permission from Bourland et al., 1988).

Bourland et al. (1987) illustrated the effects of hot water treatments in relation to the seed quality curve by comparing germination after hot water treating seed produced in 1984 and 1985 (Fig. 3). Although the germination means for the 1984 and 1985 seed were at different points on the curve, the average effects of increasing treatment time from 30 to 40 min. were similar. The additional 10 min. of hot water treatment consistently decreased germination by approximately 10% in each year. By assuming that the relative effects of the 0- and 30-min. treatments were the same each year, the mean for the untreated 1984 seed would be the point to the right of the seed quality curve apex.
Because variation among genotypes for germination after hot water treatment was found to be essentially equal to variation in water imbibition rate, response to hot water treatment was closely associated with the seed coat (Bourland et al., 1987). Furbeck et al. (1993b) found that heritability of germination percentage after hot water treatment was high and collinear with water imbibition rate. Germination percentage of non-deteriorated seed had a heritability of zero. They proposed that resistance to deterioration might be genetically improved by selecting for viability after pre-treating seed from early segregating generations with hot water. Hard-seededness could be avoided by evaluating imbibition rate of resulting strains.

**Hard dormancy.** Hard dormancy is a cottonseed quality phenomenon that occurs rarely in certain seed producing areas, notably West Texas. Seed expressing this phenomenon violate the above seed quality principles, and do not respond to seed deterioration methods. Seed possessing hard dormancy produce high tetrozolium, but low germination (warm and cool) test results. In contrast to genetic hard seededness, hard dormancy seed imbibe water at a normal rate. The phenomenon does not appear to be limited to certain genotypes. Specific cause of the phenomenon and means of breaking it are unknown. N. Hopper (Texas Tech University, personal communication) suggested that hard dormancy might be related to a germination inhibitor in the seed coat, which is imbibed into the embryo as the seed imbibes water. When detected, dormant lots should be discarded for planting seed purposes or stored and not commercially planted until dormancy breaks.

**SEEDLING VIGOR**

**Seedling Vigor Defined.** Cotton is generally regarded as having low seedling vigor relative to other crop plants. Seedling vigor was not essential for survival and little natural selection for vigor occurred as cotton evolved in tropical/subtropical regions as a perennial shrub. Harsh conditions during the seed emergence and seedling stages accentuate problems associated with low seedling vigor. Cotton is frequently planted early (when harsh conditions are likely) to promote early maturity and avoid many late-season adversities. Because low vigor in seedlings could be a symptom of several interacting causal factors, the probability of improving vigor by selecting directly for vigor is low.

![Figure 3. Response of cotton seed produced in 1984 and 1985 to hot water treatments in relation to the seed quality curve (reprinted with permission from Bourland et al., 1987).](image-url)
Tall, large seedlings are generally regarded as being vigorous. However, Bailey and Bourland (1986) found no differences in the height of 17-d-old cotton seedlings in the presence (most secondary roots pruned) or absence (no secondary roots pruned) of trifluralin. Additional evidence of the fallacy of direct association of seedling size and vigor has come from evaluation of seedlings in cotton strain tests conducted at Mississippi State University in the mid-1980s (unpublished data). For several years, breeding lines showed significant and often wide variability for fresh weight, dry weight, and height of approximately 3-wk-old seedlings. However, these seedling measurements were seldom correlated with harvested yield. Thus, either seedling vigor is not important relative to yield or seedling vigor should be redefined. This experience led to defining seedling vigor in cotton using three criteria: 1) well-developed seedling roots, 2) rapid true-leaf differentiation, and 3) low levels of seedling disease.

**Well-developed Seedling Roots.** Intuitively, an increase in the inherent ability to produce secondary roots should enhance drought tolerance and nutrition status while lessening the adverse effects of agents that reduce root growth. Work with the herbicide trifluralin provided an opportunity to study secondary roots. Trifluralin is a dinitroaniline herbicide and acts as a mitotic poison to prune secondary roots in treated soil. It is normally incorporated in the top 5 cm of soil prior to planting. Cotton seedlings should escape damage of the herbicide by placement of seed in the lower portion of the treated soil. As seed germinate, seedling roots grow and develop in untreated soil.

In the late 1970s, cotton yields appeared to stabilize or decline throughout the U.S. cotton belt, and this problem coincided with the introduction and wide use of incorporated herbicides (Meredith, 1982). If trifluralin were associated with the decline, then genetic tolerance to the herbicide would be valuable. With this premise, work was initiated to select for tolerance to trifluralin using secondary root development as the selection criteria. Bailey and Bourland (1986) confirmed that seed quality must be standardized or controlled before the effects of trifluralin on secondary roots could be evaluated. An initial survey of 14 cotton cultivars indicated a wide variation for number of secondary roots in both trifluralin-treated and untreated soil (Bourland et al., 1981).

In subsequent work, increased number of lateral roots was selected by planting seed in cups filled with trifluralin-treated soil (Bourland and White, 1984). Seed were tip-germinated to normalize variation in germination speed, then placed in cups with the mycopylar end pointed to the cup’s edge (Fig. 4). Subsequent roots grew along the inside of the cup and could be exposed by removing the cup. Seedlings of lines that were selected for increased secondary root development in trifluralin-treated soil had more secondary roots and were shorter than seedlings of their parents in both laboratory and field tests (Bourland et al., 1985). These data suggested that the procedure was selecting individuals with greater genetic propensity to produce secondary roots rather than selecting for specific tolerance to trifluralin. This suggestion was confirmed by comparing root development of nine cultivars in untreated soil and soil treated with either trifluralin or a different dinotroaniline herbicide (Mitchell and Bourland, 1986). The primary factor determining secondary root production in treated soil was the cultivar’s potential to produce secondary roots in untreated soil. Vieria et al. (1995) found that number of secondary roots in either trifluralin-treated or untreated soil had high, general combining ability and high heritability. Direct selection should be an effective means to increase secondary root development. The role of the treated soil appeared to be to increase selection pressure for secondary root development. The relationship of number of lateral roots on cotton seedlings to mature plant root structure and to effectiveness of root hairs is not clearly understood.

![Figure 4. Technique for selecting increased number of lateral roots by planting tipped seed in trifluralin-treated soil then subsequently removing cup to expose roots (Bourland and White, 1984).](image-url)

**Rapid True-Leaf Differentiation.** Producers frequently speak of cotton seedlings that come up and “just sit there.” Obviously, the seedlings are not just sitting there, but should be developing roots and differentiating their first true leaves. Cotton seedling vigor might be improved if genotypes could be developed that differentiate their first true leaf more rapidly. With this focus, attention was given to the timing of true-leaf development during routine screening of cotton seedlings. From one cross (designated as
8304) of two experimental lines, several seedlings were found to have a well-developed visible true leaf at emergence (VTLE) (Fig. 5). The 8304 cross combined two experimental lines: 7803-3-5 (derived from crossing DES 56 and TX-MAR-22-74) and 7823-1-3 (derived from crossing DES 24 and TX-LE-68-73). Seed from the individual VTLE plants produced lines having as high as 31% of seedlings expressing this characteristic. A second cycle of selection produced one line having more than 60% expression (Ortiz and Bourland, 1990). Although not exclusive to this one cross, VTLE did not exceed 10% of seedlings in any of 66 other genotypes examined. True-leaf development was genetically controlled by additive variance, but with some reciprocal effects associated with seed size of the female parent (Ortiz, 1992).

In an initial field test, the VTLE trait provided a significant increase in yield with an accompanying increase in main axis nodes (unpublished data). Ortiz (1992) later found similar results in greenhouse tests and in one of three field tests. His data suggest that the VTLE in cotton can be enhanced by direct selection but could be restricted by reciprocal effects and low penetrance in available genotypes. Efforts to repeat the positive yield effects of VTLE in field tests have been elusive due to the transient and subjective nature of the trait, its low penetrance, and the inherent low relation between seedling traits and yield.

The basic premise associated with VTLE is to ignore seedling size and select seedlings that develop quickly beyond the vulnerable seedling stage. By shortening the time that the plant remains a seedling, many seedling problems can be lessened or escaped. Ortiz and Bourland (1999) found that VTLE plants had more nodes, photosynthetic area, and dry weight than normal phenotypes at 10, 20, and 30 d after emergence. A recurrent problem with field evaluation of the VTLE trait is being able to make evaluation of seedlings as they emerge. The VTLE trait is noticeable for only a short time, and then becomes masked by the further development of true leaves.

Seed of the 10 VTLE lines (all derived from the 8304 cross) were increased in 2014 and tested for VTLE in a 2015 greenhouse planting. Good expressions of VTLE were observed in selections made from one line, Ark 8304-54-06. No VTLE plants were found in the check cultivar DP 393 or in an Ark 8304-54-06 line selected for absence of VTLE. Most of the 8304 derived lines have consistently shown relatively high VTLE expression, but none have produced acceptable yield and fiber properties. When examining these VTLE lines in the 2015 greenhouse tests, we found a low occurrence of the VTLE trait in a newly released cultivar, UA48 (Bourland and Jones, 2012). Consequently, a 2016 seed increase block planting of UA48 was examined for VTLE. Out of more than 8,000 seedlings examined, we found 63 VTLE plants. These plants were flagged, and a bulk sample was taken at harvest. In 2017, a planting of this bulk produced VTLE plants, which were flagged, and the most productive ones were harvested as individual plants. Analyses of fiber properties indicated that most of these selections maintained the high fiber quality of UA48. The selections presently are being evaluated in progeny rows, which could lead to an improved line possessing the VTLE trait. If such a line is developed, the value of reducing the time associated with the seedling stage as an approach to improve seedling vigor can be fully evaluated.

**Low Seedling Disease.** The third component of cotton seedling vigor is low incidence and severity of seedling disease. Cotton seedling disease is caused by a complex of seedborne and soilborne pathogens, any of which can incite the disease (DeVay et al., 1989). Combinations of more than one pathogen infecting the same plant tends to enhance symptom development beyond that associated with single pathogens (Bourland and Bird, 1975). Soilborne pathogens commonly occur in most soils and cause diseases on a wide range of crop plants. Improving resistance to seedling disease has been slowed by the complexity of its multiple pathogens and the wide range of hosts.

Seedling disease symptoms include seed rot, pre-emergence damping-off, post-emergence damping-off, and an array of sublethal expressions (Halloin and Bourland, 1981). Disease severity is greatly influenced by environmental and cultural
factors. Factors that delay seed germination and seedling growth favor seedling disease. When seedling growth is delayed, the plant is vulnerable to the pathogens for a longer period and less able to resist infection and disease development.

The complexity of pathogens and environmental factors associated with seedling disease confounds the problem of disease control (DeVay et al., 1989). Control measures include cultural, chemical, biological, and genetic approaches. Cultural techniques (e.g., plant on well-established beds and delay planting date for higher temperatures) attempt to reduce exposure to adverse conditions. Chemical techniques, both seed and in-furrow treatments, attempt to reduce inoculum densities of the pathogens. Biological control methods employ means for enhancing populations of both native and supplemented microorganisms that are antagonistic to the seedling disease pathogens (Bourland and Caviness, 1990; Hagedorn et al., 1992).

Most cotton planting seed in the U.S. are acid-delinted using either hydrochloric acid (dry acid method) or dilute sulfuric acid (wet acid method) (Pilon et al., 2016). Prior to the widespread use of acid-delinting, cotton was planted with gin-run fuzzy seed, mechanically delinted seed (done by a re-ginning process), or flame delinted seed (typically done by burning linters from mechanically delinted seed). Except for noting that fuzzy seed tended to emerge more slowly, little difference in stands or yields were found between fuzzy, acid-delinted, and mechanically delinted seed (Wilkes and Corley, 1968). Delouche (1981) described the delinting methods and compared fuzzy, mechanically delinted, and flame delinted seed to acid-delinted seed. Positively, acid-delinted seed 1) improved handling, storage, and transporting seed, 2) enabled seed grading (removal of poorest seed), 3) facilitated metering of seed from planter, 4) enhanced uniformity of seed treatment applications, and 5) lowered seedborne diseases. Negatively, acid-delinted seed had higher processing costs, increased environmental and safety concerns, shorter storage life, and slightly lower emergence (along with faster water imbibition and germination). A new mechanical delinting method, which uses plastic brushes to abrade linters from the cottonseed, is now being developed (Holt et al., 2017). With < 1% residual lint, seed delinted by this method should maintain the positive effects of acid-delinting, except lowered seedborne diseases. This process would likely negate the environmental and safety concerns, shorter storage life, and lower emergence associated with acid-delinted seed.

Currently available fungicide seed treatments are highly effective in controlling seedling disease in cotton (Rothrock et al., 2012). Consequently, most fields do not have even sublethal symptoms. Yet, improved resistance to seedling disease is needed in severe harsh conditions and as a backup to the effective chemical treatments. Inheritance of resistance to seedling disease is complicated by the complexity of pathogens plus many environmental and biological interactions. Cotton genotypes that survive in a breeding program can be expected to possess at least some resistance to most seedling disease pathogens. Higher resistance to some specific seedling disease pathogens has been reported (Bush et al., 1978; Poswal, 1986). However, present levels of resistance to seedling disease, even in combination with cultural and chemical controls, can be overwhelmed with harsh environments that favor disease development.

One method that is now being used to evaluate for resistance to seedling disease is to determine stands in inoculated greenhouse plots. Seed of lines are treated with a fungicide that controls Pythium spp (but does not affect Rhizoctonia), then are evaluated for stand and damping-off in Rhizoctonia-inoculated plots. For Pythium evaluation, seed are treated with a fungicide that specifically controls Rhizoctonia and are then are evaluated for stand in Pythium-inoculated plots. Stands in inoculated plots are corrected for seed quality variation among the lines.

ATTAINING SEED AND SEEDLING VIGOR

Genetic improvement of both seed vigor and seedling vigor will be beneficial to the cotton industry. Optimum improvement can be made by independently considering both seed and seedling vigor, then combining them. A line possessing high seed vigor would have limited value if it had low seedling vigor. Conversely, the benefits of excellent seedling vigor components might be negated by low seed vigor. Seedling vigor can be improved by identifying and approaching each of the above components of vigor separately, then integrating them. Seedlings that develop prolific roots and rapidly differentiate true leaves are not vigorous if they are damaged by seedling disease. Conversely, seedlings can grow slowly and poorly even in the absence of seedling disease. By integrating these components of seed and seedling vigor, the probability of producing uniform stands of vigorous seedlings is increased. However,
harsh environmental conditions during seed germination and seedling development can overwhelm even the best approaches and methods that are presently available. Therefore, additional work is needed to enhance the inherent levels of seed and seedling vigor in cotton and to refine management practices for full expression of vigor.

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