ABSTRACT

CRISP, MARISA CARMEN. Impact of Visual Mechanical Damage on Cotton Seed Quality and Cotton Production in the Southeast United States. (Under the direction of Lori Unruh-Snyder).

Evaluating visual mechanical damage (VMD) and its presence and impact on cotton (*Gossypium hirsutum*) production has the potential to positively impact growers and contribute to higher yields. Cotton seed quality significantly impacts crop yield, with VMD posing a major threat by compromising germination, seedling vigor, and stand establishment. While there is knowledge of VMD within the cotton seed industry, there is no standard for measuring VMD and its severity and impact on production.

The primary objective of this project was to determine how much VMD is present in commercial seed, develop additional methodologies to evaluate seed quality, and determine which degree of VMD severity is most likely to influence stand establishment. To achieve this, an adjusted warm germination VMD test and the visual mechanical damage classification test (VMDCT) were created. The tests were validated using experimental replications in a laboratory setting using standard germination seed quality practices established by the Association of Seed Analysis (AOSA) with adjustments.

Three commercially available seed samples were hand-sorted, photographed, germinated, and classified on a vigor scale. Visual mechanical damage in commercial seed bags ranged from 4% to 33%, with very damaged seeds exhibiting the highest mortality rates.

A comprehensive survey conducted in 2021 evaluated 41 commercially available cotton seed varieties from seven companies, encompassing 168 individual seed lots across 198 samples. Companies reported VMD ranging from 3.2% to 19%, while the study found VMD ranging from 5.3% to 26.7%. A new warm germination method (VMD warm germination) was developed to

correlate seed damage with overall vigor, identifying significant correlations between VMD parameters, seed size, and oil percentage with stand counts.

Further research assessed the impact of mechanical damage location and severity on germination over 12 days. Seeds were categorized using the VMDCT and were evaluated under cool and warm conditions. Emergence was monitored on days 4, 7, and 12, with seedling vigor assessed based on AOSA vigor scale criteria. Significant differences were observed in germination rates and seedling vigor among damage categories.

The findings that severe VMD causes a significant decrease in germination highlight the importance of assessing and minimizing mechanical damage in cotton seeds to enhance germination rates and seedling vigor, ultimately improving crop productivity. A stepwise regression was conducted to determine the best VMD parameters that correlated with stand counts and the VMD warm germination percentage with seed size for stand counts at 7 DAP % and the VMD warm germination and oil percentage for stand counts at 10 DAP %. This information was used to determine which seed quality factors were the strongest predictor of stand counts and performance, giving growers more information about the seed to be used when selecting a variety. Implementing improved seed processing practices based on these insights can lead to better field performance and higher yields for cotton growers. Providing comprehensive information on damaged seeds in commercial seed bags will help growers select the best seed or seed lots for their specific conditions, optimizing their planting strategies and achieving higher overall crop yields.

This project was among the first to investigate VMD patterns in cotton seed coats as an alternative way to evaluate cotton seed quality. The key to this was evaluating relationships between cotton seed VMD and other known factors impacting seed quality, including germination rates, seed size, oil content, seedling length, and location of origin.

Impact of Visual Mechanical Damage on Cotton Seed Quality and Cotton Production in the Southeast United States

by
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DEDICATION

This thesis is dedicated to my amazing and loving parents, Diana and Todd Leggett, my brother Isaac Crisp, my incredibly supportive boyfriend Wesley Turner, and countless friends for their constant support along the way giving me the inspiration and strength to finish.

BIOGRAPHY

Marisa Carmen Crisp was born September 2, 2000, in Greenville, NC. Prior to her time at North Carolina State University (NCSU), she attended the North Carolina School of Science and Math, where she took an Introduction to Plant Sciences Technology course with Jon Davis. From then on, she fostered a love for the plant sciences and agricultural research, leading her to a B.S. in Crop and Soil Science with a concentration in Agronomy and a minor in Plant Biology in 2022. Marisa was an undergraduate research assistant working in the NCSU seed laboratory, starting her work with seeds on industrial hemp and later focusing on cotton seed, specifically cotton seed damage and vigor. This work led her to pursue a M.S. in Crop Science under Dr. Lori Unruh-Snyder. Marisa enjoys spending time with family and friends, being outdoors, fiber art, and reading.

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CHAPTER 1

REVIEW OF LITERATURE

Upland Cotton Seed

Upland cotton is primarily grown in the southern part of the United States, an area that is commonly known as the cotton belt. The vast majority of cotton grown in the US is for fiber, with seed being a secondary product. Due to this, most breeding practices are focused on fiber quality and improving yields. In some cases, due to the focus on fiber quality improvement, there is a concurrent decrease in the quality of the seed harvested from that same crop.

However, with about 21% oil and 23% protein by weight in the seed, cotton is also the fifth largest oil crop in the world and the second most important potential source of plant proteins (Liu et al., 2012). Cotton seeds are used in various products, from animal feeds to toiletries and cosmetics (Cotton Incorporated, 2024). This diverse utilization of the seed elevates its potential during the breeding process, leading to a desire for the industry to focus on quality seed that can be used as an additional tool for profit rather than a bioproduct of production.

The upland cotton ovules are composed of an immature seed (embryo and seed coat) and developing fiber. Due to the fact that fiber arises from the seed coat, fiber and seed compete with sinks fed through a common source (Kloth & Turley, 2010). Due to continual efforts to breed cotton for increased fiber yield, several seed/fiber compositional properties have likely shifted over the decades (Dowd et al., 2018). Some of the concerns developed have been smaller seed size, weaker hulls, increased seed and hull fragment contamination of fiber and reduced seed oil and protein levels (Dowd et al., 2018). Lower-quality seeds are also more prone to moisture uptake and oil degradation during storage, which causes a direct loss of extractable oil and oil refining problems (Dowd et al., 2018).

These changes are not without an agronomic cost due to small seeds having poorer germination and emergence. Small seeds also cause post-harvest problems: small seed will often pass through the gin with the fiber, leading to several post-harvest issues in the production of textiles and the unintentional movement of genetic material that might not be deregulated. The agronomic impact of small seeds and the economic impact of fiber make a compelling argument for understanding the physiological relationships between fiber and seed development and the physiology associated with specific events of fiber development (Kloth & Turley, 2010).

The cotton plant has a tap root system, where the growth of roots depends on water and nutrient availability and uptake. It has a complex structure among field crops with indeterminate growth habits that can be highly sensitive to environmental abnormalities. Cotton development can be divided into two main phases: (i) vegetative development (germination and seedling development, development of root systems and leaf, and canopy development) and (ii) reproductive development, including the development of fruiting sites, seed, and fiber (Rehman & Farooq, 2020).

Seedling development depends on the genetic makeup, seed quality, and environmental conditions at planting and/or during germination. Seeds with lower water content (<10%) have lower seed coat permeability. In cotton, the embryo has two cotyledons, which store lipids and protein (Liu et al., 2012). The embryo maturation stage of seed development is produced by an increase in the weight and volume of the cotyledons and the very rapid accumulation of food reserves (carbohydrates, oil, and storage proteins) required for germination and seedling growth. Storage lipids and proteins account for about 80% of mature cotton seed weight (Murphy, 1993).

Besides fiber, oil is the most valuable product of cotton seed and is widely used as a cooking oil and an ingredient in marinades, dressings, pastries, margarines, and shortenings.

Furthermore, oil is by far the most efficient energy storage since it contains more than twice the energy on a dry weight basis than can be stored in starch or proteins (Liu et al., 2012). Consequently, the dairy industry has regarded whole cotton seed as a feed ingredient with advantageous energy and dietary fiber properties required by the high-producing dairy cow (Liu et al., 2012). There is increasing emphasis on greater energy density in cotton seed through increasing seed oil content at the expense of carbohydrate concentrations (Coppock et al., 1987; O'Brien et al., 2005). Cotton seed, a readily available by-product of more valuable cotton fiber production, is increasingly recognized to have excellent potential for additional food, feed, and biofuels for developed and under-developed countries (Liu et al., 2012). This will improve its value with an increased focus on maintaining healthy quality seeds throughout the growing process.

Cotton Seed Quality

Cotton has long been grown in the southern United States, with more and more technology and research devoted to trait development to help preserve yield potential and improve yield and fiber quality. A factor that needs to be addressed more is the seed quality being planted nationwide. In recent years, seed quality has continuously decreased to a level noticeable to the growers. Many factors impact the quality of the cotton seed, both abiotic and biotic, ranging from the physiology of the seed to the storage of the seed. The main factors studied to determine seed quality include seed size, germination rates (Ferguson et al., 1991), seedling vigor, chemical composition (amino acids, fatty acids) (Gough, 2020), and relative damage to the seed. However, when reporting quality standards on the seed tag, not all information is readily available and presented to the grower; that information also differs from state to state based on legislation on seed quality.

Although cotton production has an incredible array of advanced technology within production and seed the need for an improvement in the quality of cotton seed has been perceived as a current and serious issue. Good-quality seed of improved varieties is one of the critical inputs for attaining high cotton yields with more economic benefits (Rehman et al., 2020).

Harvest, Ginning, Storage, and the Impact on Cotton Seed

The decision to pick highly relates to the physical and physiological maturity of the crop during the dry and hot months of August and September. After harvesting, seed cotton needs to be dried below 12% to avoid physical damage to the seed coat during ginning (Rehman et al., 2020).

Regarding cotton seed production, the most significant impact on the seed happens post-harvest in the processing and storage of the seed. Harvesting during optimal conditions for seed health would be during a hot, dry day when as little moisture as possible is present in and around the seed.

The gin is a pivotal part of the production process, removing the fiber or "lint" from the cotton seed. Cotton is a cellulose-based fiber that grows directly from the coat of the seed. The lint is often removed with saws that remove the fiber, and the seeds are soaked in an acid solution to break down any remaining lint left on the seed coat. The seed coat can be damaged during these processes, impacting its future performance if used for planting. Damaged seed does not affect the industry sectors of oil or animal feed, but it is important to understand and prevent when cotton is planted for seed.

One study done on the impact of ginning on cotton seed and fiber quality is the publication by Whitelock et al. 2019. The study examined fiber length reductions, short fiber content, fiber neps, and cottonseed damage. Each is associated with five saw gin stands of differing production rates, design, and manufacture that represent a range of gin technologies developed since the 1960s. The goal was to identify technology from those earlier designs that may guide future gin stand research and improvements to prevent loss of value (Whitelock et al. 2019). This work focused on the mechanical nature of cotton production rather than the plant itself, expanding on the relationship between different areas of cotton production related to fiber and seed quality. The mechanical relationship between harvest and processing is less often examined than the biological relationship between cotton and its environment. An understanding of both can present opportunities for making more well-rounded decisions regarding seed planting.

Whitelock et al. (2019) found that cottonseed from the gin stands with the lowest capacity had the highest residual linter content and the lowest overall seed damage aligned with the seed's short fiber content. In this case, much fiber is left on the seed coat. When the fiber is not entirely removed from the seed coat, there is less chance of damage because the blades do not impact the seed coat as closely. Also, the study indicated that historical gin stand technologies and relationships between gin parameters such as ginning rate per saw, saw spacing, seed roll density, and fiber and seed quality need further investigation. Changing practices over the years to ensure adequate fiber quality may negatively impact seed quality, increasing the rate of visual mechanical damage found on the harvested seed.

After reviewing several studies, seed damage was related to increased diameter and ginning rates (Whitelock et al., 2019). The focus on mass production and removing cotton from the seed decreases the chance of healthy seeds surviving the ginning process to get to planting. Whitelock et al. (2019) also reviewed the patterns of cotton seed damage, relating them to how the damage was caused to the seed. It stated that seed damage consisting of slits and cracks parallel to the minor axis of the seed has been characterized as conveying damage, while slits and cuts parallel to the major axis of the seed have been defined as damage due to the gin stand (Whitelock et al., 2019). Knowing the point at which a seed was damaged is a good way to determine where in the process adjustments can be made in order to decrease the rate at which VMD occurs when ginning cotton. A solution to help mass ginners prevent such damage is to run standard tests on all machines so that the rate of damage can be managed and decreased.

Seed damage typically results in seed coat fragments (SCF), which can result in additional neps (fiber entangled around an SCF) and higher trash content (Whitelock et al., 2019). Improvements in the ginning industry have increased the amount of fiber collected from the seed more efficiently. Still, there is limited information on how this has affected fiber or seed quality, particularly nep formation and SFC (Whitelock et al., 2019).

Mechanical damage to cotton seed can happen at various processing stages as the seed moves from field to bag. The nature of damage arising in seeds from the impact of pneumatic conveying installations, grate-saw-bar cleaners, gins, and linters has been studied (Mukhametshina & Rustam, 2021). In addition to the total damage to the seeds, the percentage of seeds with specific types of damage was determined for each sample, and as the raw cotton moves along the process, the total damage to the seed increases.

Delinted cotton seeds are produced by ginning machines, de-linting machines, centrifugal rollers, hoists, polishing machines, and other processes of cotton seed processing, which can easily lead to many damaged seeds (Liu et al., 2022). The traditional manual and machine vision screening methods have the problems of low efficiency, poor accuracy, unrealizable real-time monitoring, and inability to identify damage to the population of cotton species (Liu et al., 2022).

This study used Hikvision mv-ce120-10uc USB 3.0 CMOS to effectively monitor damaged seeds through processing (Liu et al., 2022). With advanced imaging technology, critical points of damage potential throughout the ginning process can be realized and altered accordingly.

Additionally, adequate seed storage can lead to better use of both natural and economic resources when seeds of poor quality are planted (De Vitis et al., 2020). Adequate seed storage relies on slowing down the seed's normal metabolism as much as possible without incurring damage (De Vitis et al., 2020). Seed longevity measures how long seed can be stored and remain viable under certain conditions. Seed longevity in storage varies significantly among species. It is also determined by the cumulative effect of the environment during seed maturation and harvesting, as well as the time of seed harvest (De Vitis et al., 2020).

Sudharani and Padmasri (2014) studied cotton seed storage and evaluated seed vigor based on storability and field performance. The study used five cotton hybrid seed lots (Bharat BT-1) with differences in initial vigor to identify and standardize the methods for assessing cotton's seed vigor and storability. The seed samples were subjected to accelerating aging for 48, 72, and 96 hours at 41 °C and 90% relative humidity. The data was collected for all seed quality parameters initially, and a germination test was carried out after subjecting the samples to accelerated aging tests (Sudharani & Padmasri, 2014). The data collected included germination %, germination speed, seedling length, vigor index, and field emergence. Correlation studies were carried out and observed that the final count of germination was suitable for assessing the seed vigor and accelerated aging for 48 hours to estimate the storage potential of cotton seed lots (Sudharani & Padmasri, 2014). The final count of germination was found to be a standard method for assessing the seed vigor and the accelerated aging for 46 hours at 90% relative humidity, and 41ºC was suitable for evaluating the storability.

Although genetic purity analysis and germination testing are priorities in estimating seed cultivar potential, vigor tests identify the lots vulnerable to less than optimum soil conditions, extremes of temperature, and moisture. Vigor differences in the seed are significant to crop establishment (Sudharani & Padmasri, 2014).

Jianhua and McDonald (1996) evaluated the appropriate method for assessing seed vigor and detecting storability through accelerated aging tests. An accelerated aging test was performed in transparent plastic germination boxes (11 cm x 11 cm x 3 cm), in which seeds were evenly distributed in single layer upon a metallic screen fastened in their inner part to hold the seeds; and then at the bottom of each germination box were added 40 mL water or saturated NaCl (Jianhua & McDonald, 1996). Immediately after, the germination boxes were lidded and kept in an incubator chamber, set at 41 ºC for 48, 72, and 96 hours. The field emergence and seedling vigor index (SVI) were recorded. It showed that accelerated aging for 48 hours would be appropriate for the estimation of the storage potential of seed lots. On assessing seed physiological potential through traditional, accelerated aging tests, other researchers have also verified a reduction in the vigor of cotton seeds during storage, especially under non-controlled environmental conditions (Sudharani & Padmasri, 2014).

In cotton, seed deterioration occurs rapidly under non-ideal storage conditions, including high moisture and high-temperature storage. Ideal conditions should be maintained well from harvest to the next sowing season without appreciable loss in vigor and viability (Narayanan et al., 2012). Cottonseed is hygroscopic and, therefore, absorbs moisture from or gives up moisture to the surrounding air. Storage temperatures below 60°F and 10% moisture content wet basis (mc w.b.) provide the best storage conditions (Cotton Incorporated, 2024).

In another study, the seeds were evaluated initially and at monthly intervals up to 10 months for their seed quality parameters via speed of germination, germination percentage, seedling length, dry matter production, and vigor index. Narayanan et al. 2012 revealed that the linted cotton seeds treated with halopolymer at 4 ml kg-1 and stored in an aluminum container maintained their vigor and viability for up to 10 months over other treatments and control. The germination percentage decreased with an increase in the storage period, irrespective of the crops, from 88% to 50%. The decline in germination over storage periods is due to the depletion of food reserves. Seed deterioration, as evidenced by the loss of viability, is associated with decreased growth of roots and shoots. The root length is a good criterion for assessing seed vigor. In the present study, the root and shoot length of the seedling showed a significant reduction over storage periods, irrespective of the treatment, container, and varieties (Narayanan et al., 2012).

Another factor that decreases viability throughout storage periods is damage to the seed coat within the bags of stored seed. Damage to the seed coat allows space for pathogens and faster degradation of the vulnerable embryo.

Seed Germination and Visual Mechanical Damage

Seed germination is impacted by the seed coat and its ability to protect the embryo and successfully imbibe water. Cotton seed germination in a controlled warm or cool germination test is measured by the length of the emerging hypocotyl (AOSA 2023). The higher the germination rate in the lab, the higher the seed quality. Many factors impact the germination rate, including mechanical damage to the seed coat. The relationship between visual mechanical damage (VMD) and cotton seed germination and vigor has primarily been excluded when considering cotton seed quality.

The first reference found reporting the methodology to determine cotton seed damage was found in the publication by Douglas et al. (1965, 1967), which discussed laboratory methods that might be used to determine the severity of VMD. Douglas et al. (1965, 1967) studies were mainly done in response to the rapid mechanization of cotton production, the development of the mechanical harvester, and the more modern adaptations of the cotton gin (Holley, 2010). This mechanization led to an 80% increase in the risk of damaging the cotton seed through harvest and production (Holley, 2010).

Douglas et al. (1965, 1967) used 100 subsamples of acid-delinted seed to determine what cotton seed damage had occurred from the mechanical harvester. The grades for damaged cotton seed at this time were: (0) No visible damage; (1) Seed coat scratched or bruised but not broken; (2) Seed coat broken only slightly but embryonic portions of seed not visible through break; (3) Seed coat broken sufficiently so that portions of cotyledons or other embryonic parts were visible through break; (4) Large section or all of seed coat removal or nearly broken seed. It was concluded that as the level increased from 2 to 4, the germination rate decreased, and germination rates were highly negatively correlated with germination percentage at 20℃ and 30℃ (Douglas et al., 1965). Damage grade was positively associated with a reduction in the seed's vigor as measured by Wiles' (10) method (Table 5). The regression equation showed that for every unit increase in damage grade, there was a corresponding increase of 20% in the difference between germination percentage at 30°C and 20°C (Douglas et al., 1965).

By 1972, The Agriculture Research Services found the incidence and severity of mechanical damage in seed lots were determined by visually examining a randomly selected group of 200 seeds after they had thoroughly gone through the delinting process. This included soaking approximately one-fourth pound of gin-run, mechanically, or flame-delinted seed concentrated in sulfuric acid. The seed was held in the acid in a woven wire dipper (strainer). When the linters were adequately dissolved, the seeds were removed from the acid and washed thoroughly under running tap water (2 minutes). They were then washed in a sodium carbonate solution for about 1 minute to neutralize them further. Finally, the seeds were dried in a small forced-air dryer. After the seeds were dried, they were processed in a South Dakota pneumatic laboratory blower at air setting 80 to remove trash and immature seeds. Those seeds were then classified into four categories: (1) noncut- seed with completely intact seedcoats; (2) pinhole damage- seed with seedcoats cracked or cut but not severely; (3) damage primarily to the chalaza end or on sides; (4) major damage- seed with large cuts or ruptures in seedcoat, part of seedcoat missing, cotyledons often exposed, and damage to radicle end of seed; (5) immature seed- seed with a light tan or whitish seedcoat and shriveled. Immature seeds were not included in the damage categories but were evaluated in some studies. The ARS (1972) concluded that the minor damaged seeds may germinate but are generally of low quality and that major damaged seeds usually do not survive the delinting process.

The visual mechanical damage test by McCarty and Baskin in 1978 classified the damage similarly to The Agriculture Research Services (1972). The main difference in the study was changing the word noncut to no damage for the first category and using magnification to classify damaged seeds. In addition to the VMD test, McCarty and Baskin (1978) reported several other rapid evaluation tests to determine seed quality. Several tests are done on cotton seed initially to ensure seed health; those tests have changed in thoroughness and expectation throughout the production system. At the time of publication, the standards used to evaluate seed quality included rapid evaluation tests that included cutting, visible mechanical damage, free fat acidity, seed coat maturity, measuring the seed temperature, and seed moisture content (McCarty & Baskin, 1978).

In 2019, the issue of a decline in cotton seed quality was reported extensively by farmers to Cotton Incorporated in Cary, North Carolina, who in response assisted the Extension Specialists of the Cotton Belt in conducting a national seed quality survey during the years 2020 and 2021, which included reviewing the past methods published on how to calculate VMD and how to replicate those methods to adapt those methods for consistency reporting VMD percentages (Crisp & Unruh-Snyder, 2023). Thus, Crisp and Unruh-Snyder (2023) organized and reviewed all past protocols for VMD, reviewed serval seed lots from 2020 and 2021, and adapted the previously reported VMD categories. Crisp and Unruh-Snyder utilized four main categories: (1) normal seeds will have no visual imperfections; (2) pinhole, a very small symmetrical hole in the seed coat; (3) damaged, a crack or abrasion on the surface of the seed that does not reach the interior; and (4) very damaged, exposure of the embryo. In addition, Crisp and Unruh-Snyder (2023) developed a catalog of patterns depending on the positional locations on the seed coat. They found that due to the nature of cotton seeds, damage to specific locations of the seed can impact the growth of the seedlings in different ways. In addition, Crisp and Unruh-Snyder (2023) found that the extent of damage varies considerably among seed lots, and removing all damaged seeds while processing them for planting would be nearly impossible.

The relationship between VMD, cotton seed germination, and vigor has primarily been excluded when considering cotton seed quality and needs to be addressed. Visual mechanical damage has been found in all seeds produced and sold in the US and will continue to impact growers through lower stand establishments and yields (Crisp & Unruh-Snyder, 2023). Still, by

2023, the AOSA’s rules and procedures handbook does not reference how to determine VMD, and VMD is not currently reported on the seed tag during the commercial sale of cotton seed.

Seed Physiology and Impact on Seed Performance in the Field

Germination in cotton starts with water and oxygen absorption through chalaza, followed by radicle protrusion through the micropyle. The radicle grows deeper, forming the tap root, which helps with nutrient and water uptake. The hypocotyl extends, forming an arch. The hypocotyl elongates and pulls the cotyledons out of the soil, which exposes the epicotyl and apical meristem, followed by the development of true leaves (Rehman et al., 2020).

Cotton seeds' mass and oil content are positively associated with seedling vigor. Seed oil content positively correlates with seed cotton yield (Awais et al.,, 2021). In contrast, seed size has been negatively associated with lint yield, as smaller seeds result in higher lint percentages. This study addressed the hypothesis that planting seed mass and total oil plus protein calorie content of cotton seeds would strongly predict seedling vigor across most field conditions. That seed impact on yield would depend on the yield environment (Rehman et al., 2020).

Although lint percent was positively correlated with lint yield in every environment, planting seed mass and calorie content were not correlated with lint yield in four of the five environments tested or when cultivar means for lint yield and seed characteristics were averaged across all environments (Snider et al., 2016). Thus, it is concluded that individual planting seed mass and total energy content for oil plus protein are strong predictors of early seedling vigor.

Vigorous seedling growth soon after planting promotes stand establishment and minimizes grower risk in several ways. Stand establishment issues can be remedied by replanting, but the decision to replant is inherently costly and limits the profitability of cotton production (Snider et al., 2016). Given the importance of seedling vigor, many researchers over multiple decades have attempted to identify seed characteristics that could be used to predict vigor. In cotton, seed size, the degree of seed filling, seed density, and seed nutrient content and composition have all been proposed as indicators of seedling vigor (Snider et al., 2016).

For a given seed lot, the total oil and protein content per seed may represent a strong indicator of vigor, regardless of whether variation in seed and seedling characteristics are genotype and environment. Recent, non-destructive methods that allow for simultaneous quantitation of seed oil and protein concentration of whole cottonseed (Horn et al., 2011) may be valuable tools for predicting seedling vigor in a seed lot-dependent manner. At each location, seedling vigor was assessed when plants were in the 2–3 leaf stage of development to avoid the potentially confounding effects of site-specific differences in soil physical characteristics, which would have altered the time required to reach that stage. Snider et al. (2016) reported that individual seed mass was positively associated with seedling vigor, as was the total oil content per seed.

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CHAPTER 2

Methodology to Determine the Percentage of Visual Mechanical Damage in a Commercial Bag of Cotton Seed.

Abstract

Cotton (*Gossypium hirsutum*) seed quality is negatively affected by visual mechanical damage (VMD), decreasing the overall integrity of the protective seed coat and adversely affecting germination rates, vigor, and performance in the field. This study aims to evaluate existing and past methods of VMD to create updated procedures to determine the percentage of VMD present in commercial cotton seed. The techniques used in this study were similar to what the Agriculture Research Services completed in 1972 in their investigation of mechanically damaged seed, where the seed was sorted into four different classes of damaged seed: very damaged, damaged, pinhole, and normal; however, this study tracked each seed to seedling's performance to establish the relationship of germination rates and seedling vigor. Three commercially available mechanically harvested, delinted, and treated seeds were hand sorted, photographed, germinated, and individually classified on a vigor scale. Visual mechanically damaged seed was found between 4% and 33% in samples taken directly from a commercial seed bag. Survival rates were determined by the severity of the seed's damage, where very damaged seeds resulted in the highest quantity of dead seeds or seedlings. Seedlings grown from damaged seeds were significantly smaller in length and had decreased germination rates overall. Giving an estimate of damaged seed in the seed bag would provide a complete set of information to the growers to best select a variety for their circumstances. Overall, removing very damaged seeds with exposed embryos would increase germination rates and potential performance in the field.

Introduction

The objectives of seed testing are to provide critical information on the quality of the seed in terms of varietal purity and its probable physiological growth measured by the seeds' ability to germinate and establish an acceptable stand. Methods utilized for testing seed quality have been developed and validated through organizations, including the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA), and these methods are updated and published routinely. The critical aspects of establishing any method of testing seeds are consistency, repeatability, and predictability of field performance. This study aimed to create a method to determine the percentage of visual mechanical damage (VMD) seed in cotton (*Gossypium hirsutum*) that can also correlate to seed quality and vigor, like the warm and cool germination tests utilized as the standard in the cotton seed industry today. The goal was to develop a VMD method that would be adapted and recognized by the AOSA, ISTA, or other private laboratories to determine the percentage of VMD seed for cotton seed farmers and to achieve a way to report that information on the seed tag so growers can adjust their planting rate accordingly. This topic has not been studied extensively in the last 50 years, and the data reported within this research brings this topic to the forefront alongside other modernizations in the cotton industry.

In 2019, after experiencing optimal temperatures during the early part of the planting season, cotton farmers reported poor stands of some set lots of cotton seed to Cotton Incorporated in Cary, North Carolina. Immediately following, between 2020 and 2021, Cotton Incorporated supported a study to assist the Cotton Extension Specialists at North Carolina State University and across the country in conducting a national seed quality survey. This included reviewing the past methods published on calculating the percentage of VMD seeds and how to replicate those methods to adapt for consistency and reliability when reporting VMD percentages (Crisp &

Unruh-Snyder, 2023).

Seed performance complaints are undoubtedly familiar, and in 1998, the North Carolina General Assembly passed a law to help resolve seed performance issues. Growers who purchased seeds that fail to perform as labeled may file a complaint with the Commissioner of Agriculture to have the complaint investigated by the state seed board (Edmisten et al., 2024). Considering the 2019 issues with poor quality seed, a database tool was developed by the North Carolina Department of Agriculture & Consumer Services in 2020 to give growers access to both NCDA & CS warm and cool germination test results of their seed lots to assist in planting decisions (Edmisten et al., 2024).

The warm germination test is the standard and the only legal standard for all crops to determine seed quality and is reported on the official seed tag. The warm germination percentage reported on a cotton seed tag for cotton is generally 80% in the United States. The warm germination test could be higher, but the warm germination rate reported on the seed tag is most commonly 80%. The warm germination test provides the seed's potential to produce a normal seedling when planted in nearly ideal conditions. Only normal seedlings should be counted as having the potential to grow and produce a plant in similar field conditions. An element not listed on a seed tag for cotton seed is the cool germ results or the percentage of VMD. For example, the Australian Cotton Seed Association reports the percentage of VMD on the seed tag for the farmers to know at purchase (Personal Communication); however, that is not the case in the United States. A factor that can potentially contribute to a rapid decline in seed quality is the percentage of VMD in the bag (ARS, 1972). The VMD percentage is a commodity-specific test for cotton since most of the damage to the seed coat occurs during harvesting, as well as through the ginning process and the use of machinery (fine-toothed saw blades) to separate the lint from the seed.

The first reference of methodology to determine cotton seed damage was found in the publications by Douglas et al. (1965, 1967), who discussed the laboratory methods used to determine the percentage of VMD. They tested four varieties, using 100 acid-delinted seeds to determine cotton seed damage by a mechanical harvester. The damage grade reported was the following: (0) no visible damage; (1) seed coat scratched or bruised but not broken; (2) seed coat broken slightly but embryonic portions of seed not visible through break; (3) seed coat broken sufficiently so that portions of cotyledons or other embryonic parts were visible through break; (4) large section or all the seed coat removed or nearly so and broken seed. The authors concluded that as the grade level increased from 2 to 4, the germination rate decreased by at least 50%, and the link between VMD and the germination rate was negatively correlated. They also reported that the germination was reduced if the damaged seed was germinated at 20°C (cool test) compared to 30°C (warm test), indicating that the degree of VMD was accentuated under non-ideal growing temperatures, which would be expected at the temperature difference. Douglas et al. (1965, 1967) also found that for every unit increase in damage grade, there was a corresponding increase of 20% in the difference between germination percentage at 30°C and 20°C.

By 1972, the Agriculture Research Services (ARS) conducted a study testing six different varieties to quantify the incidence and severity of mechanical damage by randomly selecting 200 seeds after they had thoroughly gone through the delinting process, which included soaking approximately one-fourth pound of gin-run, mechanically or flame-delinted seed in a concentrated sulfuric acid solution. The seed was held in the acid in a woven wire dipper (strainer). When the lint was adequately dissolved, the seeds were removed from the acid and washed thoroughly under running tap water (2 minutes). They were then washed in a sodium carbonate solution for about 1 minute to neutralize them further. Finally, the seeds were dried in a small forced-air dryer. After the seeds were dried, they were processed in a South Dakota pneumatic laboratory blower at an air setting of 128 kmh to remove trash and immature seeds. The seeds were then placed into the following categories: (1) non-cut, seed with completely intact seed coats; (2) pinhole damage, seed with only one or two small punctures in seed coats; (3) minor damage, seed with seed coats cracked or cut but not severely, where damage is primarily to the chalaza end or on sides; (4) major damage, seed with large cuts or ruptures in the seed coat, part of seed coat missing, cotyledons often exposed, and damage to radicle end of seed; (5) immature seed, seed with a light tan or whitish seed coat and shriveled. The immature seeds were not included in the damage categories but were evaluated in some studies. The ARS (1972) concluded that the minor damaged seeds may germinate but are generally of lower quality and that major damaged seeds usually do not survive beyond the delinting process.

McCarty and Baskin (1978) repeated the ARS (1972) classification system with a random selection of 200 to 400 seeds for evaluation; however, they changed the word non-cut to no damage for the first category and suggested the use of magnification for the evaluation. In addition to the VMD test, McCarty and Baskin (1978) reported several other rapid evaluation tests that could be conducted on cotton seed to examine seed health, which included cutting, free fat acidity, seed coat maturity, seed temperature, and seed moisture content.

The official seed vigor testing handbook from the AOSA discusses germination and vigor and states that seeds considered germinable may vary from weak to robust in-field performance (AOSA 2009). Going into a more specific classification of the seed can lead to better explanations of performance and vigor related to field performance.

As a result, over the past 50 years, the relationship between VMD and cotton seed germination and vigor has primarily been excluded when considering cotton seed quality. To have a healthy seed, a non-damaged seed coat that has a healthy embryo inside is needed to imbibe water for growth and development. When the seed coat is compromised, the embryo is vulnerable to degradation through exposure or pests entering it. Measuring the severity of damage done to the seed coat is a way to determine the potential impact damage has on the growth and development of the seed and resulting seedling.

Throughout this study, the impact of VMD damage has been documented, and this study's primary purpose was to bring the topic to the forefront. The more damage a seed has, the less likely it is to survive to maturity and perform at the same level as a plant growing from a normal, non-damaged seed (Douglas et al., 1965; Douglas et al.,1967; ARS, 1972; McCarty & Baskin, 1978).

Materials and Methods

The study was conducted at the North Carolina State University’s (NCSU) seed laboratory in Raleigh, North Carolina, from 2020 to 2021. The seed was received in May 2020, and a sample was sent to the North Carolina Department of Agriculture & Consumer Services (NCDA & CS) for warm and cool germination percentages to compare to the source companies that reported warm and cool germination. The companies' germinations would have been conducted in January 2020. A request for information was sent to each seed company individually to establish a baseline and allow for comparisons. Based on a sample’s lot number, the information requested included warm and cool germination, seed size, and VMD. There was no attempt to determine where and when each company tested seed, though it is understood that most will test internally and with a third-party state lab. Presumably, the information shared by most companies was obtained from a third-party state lab. The companies contacted were generally responsive to this request, and most, but not all, information requested was received. Information was coded for each company and variety, and they are consistently represented throughout the manuscript.

From October 2020 to January 2021, the varieties were selected by Cotton Inc for VMD analysis at NCSU and were sorted, photographed, and germinated within this time frame. To start this study, previous methodologies were reviewed (Douglas et al., 1965; Douglas et al., 1967; ARS, 1972; McCarty and Baskin, 1978), which assisted in the design to test random batches of commercially available seeds that were delinted and treated. Two of the varieties selected were larger seeds, 4443 seeds per kg (Variety 1) and 4611 seeds per kg (Variety 3), while the third was considered a smaller seed at 5990 seeds per kg (Variety 2). The populations tested were 100, 200, and 300 seeds, with two replications for each of the three varieties. The seeds were hand-sorted into four categories: (1) normal, with no visual imperfections; (2) pinhole, a small symmetrical hole in the seed coat; (3) damaged, a crack or abrasion on the surface of the seed that does not reach the interior embryo, and (4) very damaged, exposure of the embryo (ARS, 1972) as shown in Figure 1. The VMD individual percentages were recorded in Table 1 for each variety, population, and replication for very damaged (VD), damaged (D), and pinhole (P), along with mathematical calculations of the totals of VMD (Total VMD= VD + D + P) and the calculation of (VD + D), which was calculated to help assist how similar or different the calculations were to the companies reported VMD percentages.

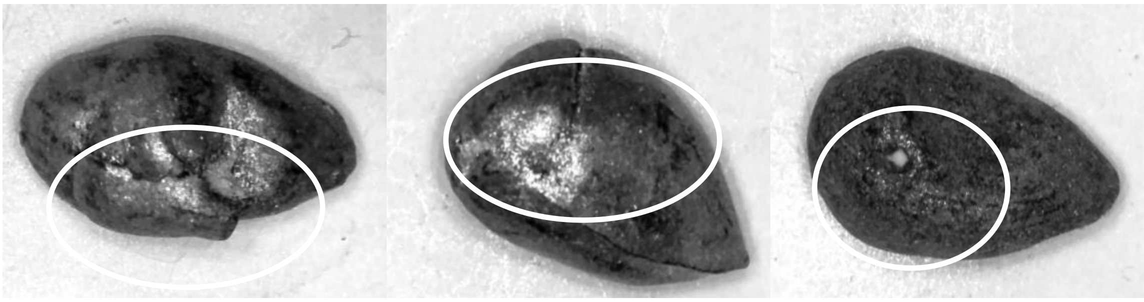

Figure 1. Examples of Visual Mechanical Damage (VMD) from left to right are examples of visual mechanical damage enclosed within the white circle. The very damaged seed (left) is a misshaped seed with an exposed embryo; the damaged (middle) has a vertical crack; and the pinhole (right) has a center hole within the white circle.

When the photographs were taken, the proper angle was achieved to capture the fact that the damaged seeds were less severe, and the pinhole seeds varied in the depth of the puncture, while the very damaged seeds were the worst. Extensive quality control training was conducted throughout this study's sorting and growing processes to ensure consistency. Verification was done by switching sample sets between graders and reviewing each sample sorted to ensure an agreement was reached over the category used and the level of damage done to the seed for each seed variety before any germinations were conducted. Thus, each set was reviewed for its correct category. This was done for each replication conducted for the 300, 200, and 100 seeds. In each set, normal (non-damaged) seeds were included in the germination procedure at the same rate as damaged seeds to ensure the presence of a control.

Before germination began, each seed was photographed with an iPhone 11 and a magnifying microscope to assist with categorizing the level of damage. Each seed was given a number to track throughout the VMD evaluation and placed in an individual container until it was germinated. Those numbers stayed with the seed through the germination process, where four seeds were in each germination box 10.16 by 10.16 cm by 3 cm, as shown in Figure 2, and the numbers were placed in each quadrant. Two layers of fully saturated germination paper were placed at the bottom of the box, seeds were placed with their appropriate number, and then two more layers of moistened germination paper covered the seeds.

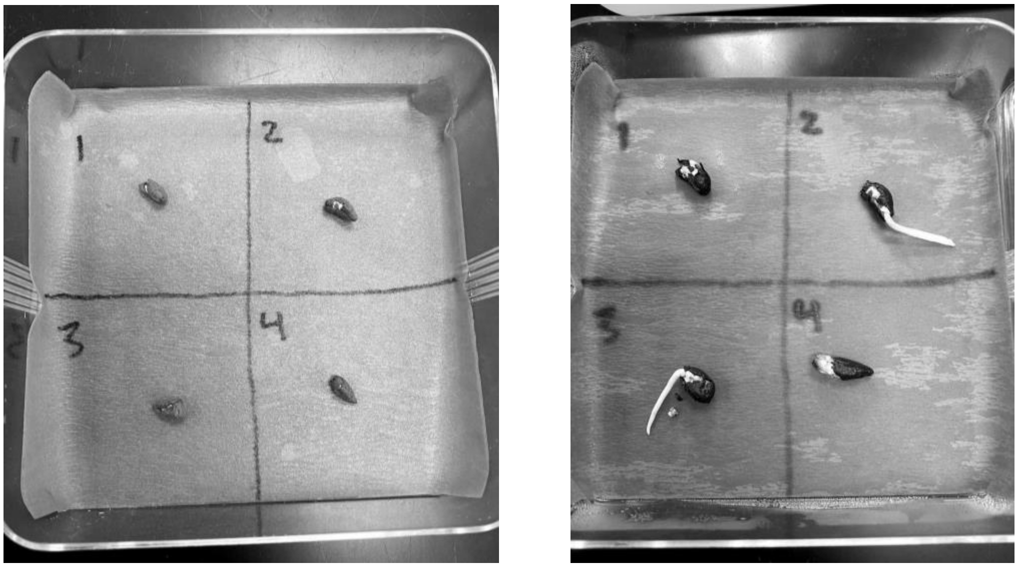

Figure 2: The seed boxes used in the germination tests were tracked with individual numbers and photographed throughout the germination process. Four damaged seeds are shown on Day 0 (left) and Day 4 (right).

The germination boxes were then stacked and placed into plastic bags to contain the moisture within them while in the germinator. The boxes held moisture; no additional water was added throughout germination. The seeds were germinated using a standard warm germination technique (AOSA 2020). They were germinated at 20-30℃ with light for 8 hours during the 30 ℃ period and dark for 16 hours during the 20 ℃ period. On the seventh day, a single final count was made. Normal seedlings are defined as those with a combined radicle plus a hypocotyl length of four cm or longer.

The seeds were photographed on days 4 and 7. The seed or seedlings were placed in the following categories based on length/appearance: strong (greater than 8 cm), weak (8 to 4 cm), abnormal (greater than 4 cm), abnormal (less than 4 cm), dead, or hard seed to categorize the vigor of each seedling. Warm germination tests are usually reported as the percentage of normal and abnormal seedlings for a warm germination test (AOSA 2020). North Carolina State Seed lab created the measurement categories 4 cm to 8 cm for weak and over 8 cm for strong for the VMD warm germination test to assist in vigor analysis. The goal was to obtain additional information to help correlate the seed damage to the seedling health or vigor. If it was abnormal, it was given a letter that corresponds to the following categories of seedling health based on standards in the Seed Vigor Handbook by AOSA (Baalbaki et al., 2009): (a) necrosis of cotyledon, (b) stubby primary root, (c) one cotyledon, (d) necrosis of hypocotyl, (e) no primary root; (f) decayed cotyledons and epicotyl, (g) no cotyledon, (h) no primary root or secondary roots, (i) damaged hypocotyl, (j) shortened hypocotyl, (k) very little secondary roots. The analyses were conducted using JMP software, Version 17, from SAS Institute Inc. The ANOVA was performed to determine if there were statistically significant differences among the mean responses of treatments between sample size and VMD categories, setting significance at the $p < 0.05$ level. Where the ANOVA showed significant differences, pairwise comparisons were made using Student's t-tests to explore specific group differences. The results are presented as means, and significant differences between treatment groups are denoted using different letters, with groups not sharing the same letter differing significantly ($p < 0.05$). A Chi-Square Test of Independence assessed the differences in categorical responses among the three varieties. This non-parametric test was chosen to determine if the distribution of response categories differs significantly across the varieties, assuming that observations are independent and adequately frequent in each category. Before analysis, data tables were prepared to count the number of observations in each category for each variety. These counts were calculated using the Chi-Square test, degrees of freedom, and associated p-values. The analyses were conducted using JMP software, Version 17, from SAS Institute Inc. The ANOVA was performed to determine if there were statistically significant differences among the mean responses of treatments between sample size and VMD categories.

Results and Discussions

The percentages of each damage category were compared between the 100, 200, and 300 populations; however, there were no significant differences (Table 1). The 300 seed population was the ideal batch size to determine a more accurate percentage of VMD, as also reported by McCarty and Baskin (1978), who found the 200 to 400 seeds acceptable for deciding on VMD. Moreover, the 300 seed population was more representative of a seed lot, and it appeared to help assess the impact on germination and its potential in the field, as reported by the evaluators. As shown in Table 1, larger quantities of damaged seeds were observed in varieties 2 and 3; the presence and severity of damage were also reflected in their germination and vigor rates (Table 2). The populations for varieties 1, 2, and 3 ranged from low to high due to the difference in damaged seeds within the populations. The more damaged seeds found in a population, the more seeds would be included in the germination study.

Table 1 shows that the total VMD ranged for the three reported varieties from as little as 4% for variety 1 (100 seeds) to 33% for variety 3 (100 seeds). In contrast, the information provided by the source company indicates that variety 1 had a reported VMD percentage of 3%, variety 2 had a reported 12%, and variety 3 did not have a percentage reported. The average rate of VMD found in variety 1 was 6.5% overall, with 4% for just very damaged seeds and 6% when including both very damaged and damaged seeds. The average rate of VMD found in variety 3 was 13% overall, with 8% for just very damaged and 11% for both very damaged and damaged seeds. Based on average comparisons, the reported VMD percentage provided by the source company is likely based on the easy-to-spot, very damaged seeds. There was no significant difference between the measurements found in the 100, 200, and 300 sets.

The findings reported in this study were like those found in ARS (1972), where the incidence and severity of mechanical damage in seed lots ranged from 11.5% to 13.5%, with decreasing rates of germination. ARS (1972) indicated that as storage periods lengthened from 0 to 6 months, the degradation of seeds increased when the seeds had higher levels of damage. Comparatively, Douglas et al. (1967) found 11% damaged seeds when hand harvesting and 46% damaged seeds when mechanically harvesting. McCarty and Baskin (1978) did not provide their findings on the exact percent damage when repeating the mechanical damage study.

Table 1. Percentage of visually mechanically damaged seeds of three varieties. Report of ANOVA for the percentage of visually mechanically damaged seeds of three varieties, and no significant difference was found at a p-value of 0.05.

Variety (V) Number of Seeds	Very Damage% (VD)	Damage% (D)	Pinhole% (P)	Normal %	VD+D+P%	VD+D%
V1-300	4.8 [a]	2.0 [a]	0.9 [a]	92.4 [a]	6.9 [a]	7.7 [a]
V1-200	2.3 [a]	2.8 [a]	1.0 [a]	93.9 [a]	5.1 [a]	6.1 [a]
V1-100	5.0 [a]	1.0 [a]	0.0 [a]	94.0 [a]	6.0 [a]	6.0 [a]
V2-300	7.4 [a]	2.7 [a]	0.7 [a]	89.4 [a]	10.0 [a]	10.7 [a]
V2-200	9.5 [a]	2.0 [a]	1.8 [a]	86.8 [a]	11.5 [a]	13.3 [a]
V2-100	9.5 [a]	3.5 [a]	2.5 [a]	64.5 [a]	13.0 [a]	15.5 [a]
V3-300	17.0 [a]	3.2 [a]	1.5 [a]	78.3 [a]	20.2 [a]	21.7 [a]
V3-200	18.5 [a]	5.0 [a]	2.5 [a]	74.5 [a]	23.5 [a]	26.6 [a]
V3-100	19.0 [a]	3.5 [a]	5.0 [a]	74.0 [a]	22.5 [a]	27.5 [a]

Table 2. Warm and cool germination percentages for the NCDA, each Company, and NCSU

	Warm Germination %			Cool Germination %		
Source	NCDA*	Company	NCSU	NCDA	Company	NCSU
Variety 1	75	92	59	53	74	29
Variety 2	88	91	71	19	68	23
Variety 3	58	88	79	41	32	24

*Abbreviations: NCDA (North Carolina Department of Agriculture, Company (seed source company), NCSU (North Carolina State University)

The percent germination is shown for each variety in Table 2. The three varieties had differences of up to 20% for warm germination and 40% for cool germination. This difference is likely due to many factors, such as genetics, location grown, differences in seed quality standards from location of origin, seed size, and time elapsed between studies performed. The germination studies were done at different times during the year, which may explain the larger differences between the study at NCSU and the other two. Data from the companies was generated in January 2020, the NCDA data was from May 2020, and the NCSU data was generated between October and January 2021. This range of dates should be considered when comparing germination rates of the varieties based on the location of germination. While this should not impact the damage rate due to the damage occurring mechanically rather than environmentally, the difference in germination time can impact warm and cool germination rates as the seeds decline further over time.

The company provided warm germination data showing differences of 1% between variety 1 and variety 2, a 4% difference between variety 1 and variety 3, and a 3% difference between varieties 2 and 3, compared to the NCSU data, showing differences of 12%, 20%, and 8%, respectively. Moreover, the NCDA data shows 13%, 17%, and 30% respectively. When considering the time that elapsed, the variety with the most germination range was variety 3 across all testing. Due to the wide range of warm germination results, it was challenging to conclude this aspect of the data when comparing it across the three locations of information since a wide range of time passed.

Varieties 1 and 3 had larger seeds, with 4443 and 4611 seeds per pound, respectively, while variety 2 had smaller seeds, 5990 seeds per pound. This may explain the difference in germination rates compared to the presence of VMD in the samples. Larger seeds tend to have a higher likelihood of damage due to their larger surface area. It was also noted that reported standards for germination suggest that initial ratings were for the first six months after seeds were fully processed.

Comparatively, the germination rate differences between varieties for ARS (1972) were found to have a difference of up to 25% when tested at the same time. In comparison, Douglas et al. (1967) found a difference of 27% between hand-harvested undamaged seeds and machine-harvested machine-damaged seeds.

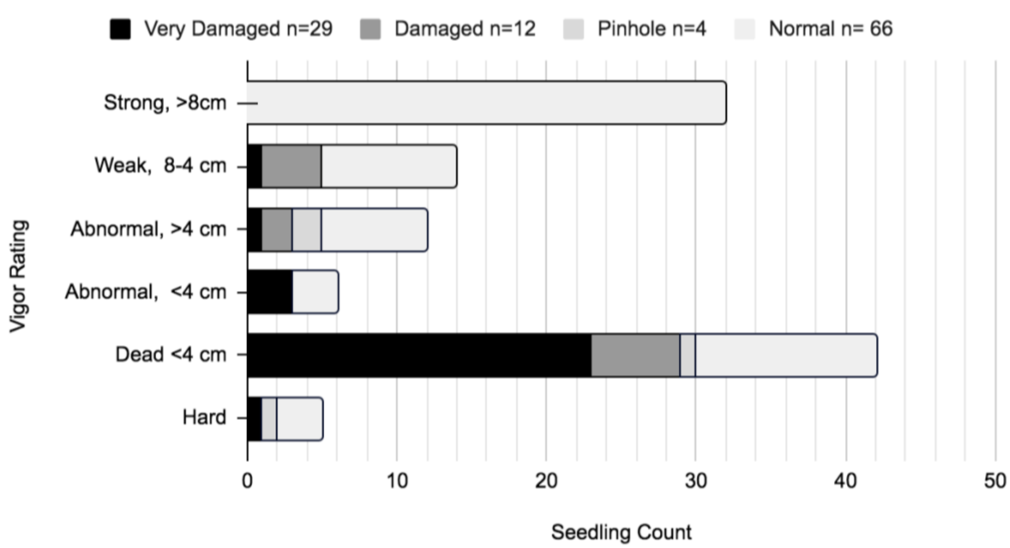

Figure 3. Seedling evaluated for Variety 1: germinated seedling vigor rating categories for tested seeds (N=111).

The VMD classification of the ARS (1972) was used for vigor ratings in cotton seedlings. The seedlings evaluated for Variety 1 (N=111 were classified for VMD by adapting the ARS (1972) method of vigor ratings in cotton seedlings. There was an increase in lower performing categories (Dead, Abnormal< 4) with a higher severity of damage rating. The highest-performing categories with the longest seedlings were mainly made up of normal seeds.

In Figure 3, most of the dead seeds (n=37) were very damaged seedlings, and most normal seeds were strong seedlings (n=48). Figure 3 illustrates that VMD seeds often develop seedlings with abnormal roots, as seen in Table 3. Preliminary data indicated that most seedlings were abnormal, and seedling root development could not withstand stress once planted (Maeda et al., 2021). The further ratings of vigor allowed more conclusions to be drawn about root health and potential, with the notation of secondary root development and overall seedling health. In Figure 4, most dead seeds (n=78) were in the very damaged category, and most normal seeds (n=110) were in the strong category, like in Figure 1.

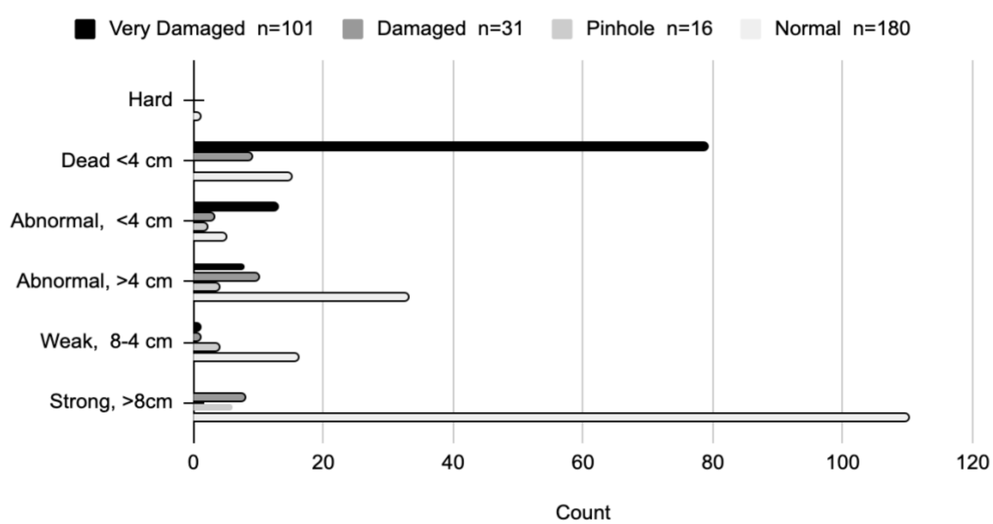

Figure 4. Seedlings evaluated for Variety 2: germinated seedling vigor rating categories for tested seeds (N=238).

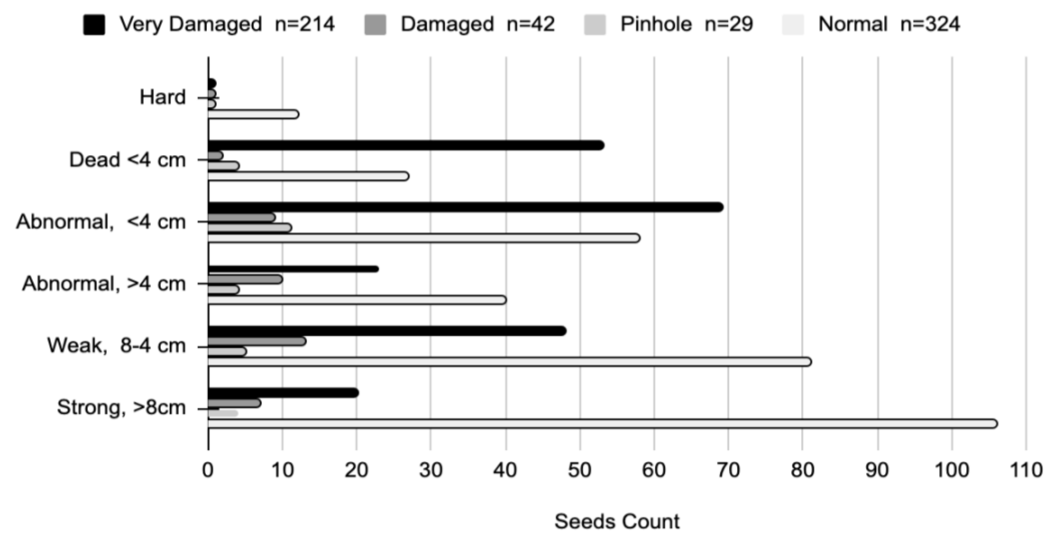

Figure 5. Seedlings evaluated for Variety 3: germinated seedling vigor rating categories for tested seeds (N=609).

In Figure 5, most dead seeds (n=52) were in the very damaged category, with a lower amount of very damaged seeds (n=20) in the strong category. Most normal seedlings were in the strong category (n=105).

The total quantity of seeds and their vigor classes were represented in Figures 3, 4, and 5. It was noted that there is a higher sample size for varieties 2 and 3 when evaluating this data. Comparing the results observed in both the germination rates in Table 2 and the vigor classifications showed that varieties 2 and 3 had higher germination rates. However, all varieties showed a similar trend of vigor distribution. In the way many of the normal seedlings were in the longer abnormal (>4 cm), weak (8-4 cm), and strong (>8 cm) categories, as well as a large quantity of the very damaged seeds, were in the lower end of the spectrum at dead and abnormal. The variety-specific differences reported could also be shown as the presence of different categories of damage, which resulted in varying levels of vigor. Figure 5 displays that the population of seeds has a broader spread throughout the classifications of vigor, whereas variety 3 displayed damage but may not have been an indicator of quality since there was a broader dispersal than the other two varieties. Looking more closely at the seedlings, they were classified across 11 categories to give more information about seedling vigor and potential in the field based on specific characteristics, as shown in Table 3.

Table 3. The incidence of vigor abnormalities of cotton seedlings within each category per variety by the Association of Official Seed Analysts and presented in the Seed Vigor Handbook (Baalabki et al., 2009).

	Incidence of Vigor Abnormalities in Cotton Seedlings											
Vigor Category (a-k)	**Normal**			**Pinhole**			**Damage**			**Very Damaged**		
Variety (1-3)*	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
necrosis of cotyledon (a)	6	16	49	1	7	2	3	2	11	1	1	34
stubby primary root (b)	7	0	1	1	0	0	4	0	0	0	0	3
necrosis of hypocotyl (d)	3	0	9	0	0	0	1	0	0	0	0	0
no primary root (e)	0	4	22	0	0	1	0	0	5	1	0	5
decayed cotyledons and epicotyl (f)	0	0	2	0	0	0	0	0	1	0	1	2
no cotyledon (g)	0	1	0	1	0	0	0	0	0	0	1	1
no primary root /secondary roots (h)	16	35	55	0	5	4	4	11	12	1	9	0
damaged hypocotyl (i)	0	0	4	0	0	0	0	0	1	0	0	29
very short secondary roots (k)	15	45	5	0	2	1	1	4	0	2	3	5

*Note: The categories for one cotyledon (c) and shortened hypocotyl (j) were not present in the data.

The range of characteristics and variety associated with each category are displayed in Table 3. There was a prevalence of seedlings that showed necrosis of cotyledons (a), no primary root or secondary root (h), and very few secondary roots (k). Normal seedlings were the category with the most incidence of necrosis, likely due to the seedlings growing to the point of requiring photosynthesis for survival and not having further access to grow and develop a more robust root system. An example of the visual deformities suffered by these seedlings can be seen in Figure 6.

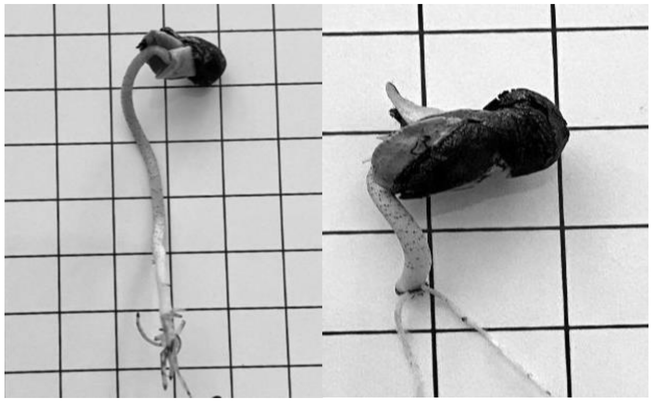

Figure 6. **Two damaged seedlings** were photographed on Day 7 in the categories (b) stubby primary root and (e) no primary root. **The seed boxes used in the germination** were tracked with individual numbers and photographed throughout the process.

Variety 3 had the broadest range of incidence (Table 3). This was not surprising since it also had the highest level of damage. Based on the vigor standards set by AOSA, the more damaged seeds present, the lower the vigor rating can be given to those seeds.

Additionally, as shown in Table 4, the length of seedlings followed the trend for all three varieties, with normal seedlings having the most extended length and very damaged seedlings having the shortest.

Table 4. The average length of seedlings evaluated for each variety for each category.

Category	Variety 1	Variety 2	Variety 3
Normal	7.8[a]	10.0[a]	8.3[a]
Pinhole	5.0[ab]	8.0[ab]	5.2[bc]
Damage	4.5[ab]	6.3[b]	7.5[ab]
Very Damage	1.0[b]	1.24[c]	4.6[c]

Note: Averages not sharing a letter within a column are significant at a p-value of < 0.05.

Based on Table 4, the average seedling lengths for the varieties had an extensive range, showing the difference in growth rate over the same period for the different damage classes. Within these first 12 days of germination, the four categories had different growth and development rates. The difference in germination rate between categories can affect stand establishment when planting these varieties in a field. If there were an 8 cm seedling difference between the best-performing seed and the worst-performing seed simultaneously, the smaller plant would be more susceptible to pest and weather damage for a more extended period. In addition, this could close the field's overall canopy slower, leading to lower stand counts, increased weed pressure, and increased risk to thrips.

When planting a field, there is likely a random distribution of damage throughout rather than a collection of all damaged seeds in one plot or row. However, an uneven establishment could prove detrimental despite the random distribution throughout a field. Weaker seedlings are not ideal, even when randomly dispersed. More information, such as average seedling length per variety, would be helpful in variety selections, indicating seedling vigor for that variety and seed lot.

The extent of damage varies considerably among varieties and removing all damaged seeds while processing them for planting would be impossible. Scratches or cracks in the seed coats that did not expose the embryonic tissues adversely affected germination and stand less than the damage than exposed the embryonic tissues. Removing the seed with the exposed embryo would be the easiest to sort out of the bag and would help decrease the rate of VMD in the seed bag.

Conclusions

Quantifying the VMD percentage in a seed bag would give a better idea of the potential impact on the growth of the cotton in the field, where a higher percentage of VMD would reduce its germination rate. In addition, photographing each seed and seedling and creating a seedling length classification system increased the ability to understand how VMD impacts seedling health. This research also started the first repository of images of cotton seed damage correlated to seedling health. This is a critical component of the first report on such methods, which follows the seed to the last germination reading day. However, more work is necessary to understand the differences in germination rates and VMD prevalence, expanding on where the damage is located on the seed and what process is causing the most harm to otherwise healthy seed.

One of the study's limitations was that sample sizes spanned a wide range that was not maintained for each variety throughout germination. To reiterate, if the sample size was 100, 200, or 300 seeds, they were reviewed individually for their appropriate damage category. Once the number of damaged seeds was found, the same amount of non-damaged seeds was selected to be germinated as the control for the number of damaged seeds. This would explain why a different population of seeds was presented in Figures 3, 4, and 5. Overall, even with a variable population, the methodology conducted to determine the VMD percentages was within range of the VMD percentages provided by the source companies.

The next best step for moving forward would be determining what damage should be removed from the cotton seed bag and whether the seeds' seed size correlates to the damage type using the image analysis system, the WinSEEDLE 2003b Image Analysis System (Regent Instruments, Inc., Quebec, Canada), specifically designed for seed morphology and analysis. Also, it would be critical to repeat these procedures using more than just three varieties in the germination process. A more extensive study looking closely at more or all commercial varieties would assist in quantifying the number of damaged seeds present throughout the Cotton Belt among the varieties selected for planting.

The relationship between VMD, cotton seed germination, and vigor has primarily been excluded when considering cotton seed quality and needs to be addressed. Visual mechanical damage has been found in all seeds produced and sold in the US and will continue to impact growers through lower stand establishments and yields (Crisp & Unruh-Snyder, 2023).

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Chapter 3

Determining the Percent of Visual Mechanical Damage in Commercially Sold Cotton (*Gossypium hirsutum*) Seed as it Relates to Seed Quality Indicators

Abstract

Planting the highest quality cotton (*Gossypium hirsutum*) seed is pivotal in achieving a high-yielding crop. The study was conducted to investigate the relationships between seed quality in the form of visual mechanical damage (VMD), seed oil, density, index, seed size, and rates of warm germination across all commercially available varieties of cotton seed as they relate to stand counts in the field to those reported by companies collected during a seed quality survey across the Cotton Belt, in 2021. In addition to validating the VMD percentages, a new warm germination method (VMD warm germination) was created to correlate how damaged seeds relate to overall seed vigor and seed damage. There were 41 commercial varieties evaluated, representing 7 companies, with 168 individual seed lots across 198 samples. The VMD found in the seed bag reported by the companies ranged from 3.2 to 19%, compared to the total VMD calculated in this study, ranging from 5.3 to 26.7%. The best linear regression closest to the company's VMD reported percentage was the calculated VD percentage with a significant R^2 value of 0.73 (p-value of $\leq$.0001) and a correlation coefficient of 0.8556 with a p-value of $\leq$0.001. A stepwise regression was conducted to determine the best VMD parameters correlating with stand counts and VMD warm germination percentage with seed size for stand counts at 7 DAP % with an R^2 value of 0.6289 and the VMD warm germination and oil percentage for stand counts at 10 DAP % with an R^2 value of 0.5493. This information was used to determine which seed quality factors were the strongest predictor of stand counts and performance, giving growers more information about the seed to be used when selecting a variety.

Introduction

Cotton (*Gossypium hirsutum*) production has seen an increase in the mechanization of harvest, seed processing, and production sectors over the past decades. Mechanization in the seed processing and production sectors has increased the seeds' potential for visual mechanical damage (VMD). Damage can allow pathogens to enter the seed embryo, inhibit germination, or reduce competitiveness compared to a non-damaged seed (Douglas et al., 1965, 1967).

In recent years, farmers in the United States have become increasingly concerned about cotton (*Gossypium hirsutum*) seed quality. These concerns specifically pertain to cases where planting conditions were deemed adequate, yet fields did not achieve acceptable stand establishment. In 2019, a nationwide cotton seed quality survey project was initiated to address these stakeholder concerns. This project represents a collaborative effort among Extension Cotton Specialists and is supported by Cotton Incorporated, a non-profit organization representing cotton growers in the United States (Maeda et al., 2021).

The notable decline in seed quality has been exacerbated by diminishing seed sizes, increasing seed costs, and the necessity for growers to cultivate larger acreages under suboptimal conditions with a uniform and acceptable stand. From a commercial perspective, the industry widely utilizes germination tests conducted under controlled conditions to determine planting seed quality. Two of the most common are the standard warm germination test (20/30 °C with 8 hours of light) and the cool germination test (18 °C with 24 hours of dark). While the current industry standard, these tests establish the maximum ability to produce viable seedlings- of a seed lot (AOSA, 2020) but do not always approximate or predict potential plant productivity in a field setting. This is because once in the field, several external biotic and abiotic factors influence germination and seedling development, as well as varying temperature and moisture extremes, making it difficult to precisely isolate and quantify seed quality's impact on final crop productivity.

The cotton industry currently regards cool germination test results (also known as the Texas Cool Test) as a good indicator of seed vigor (Smith & Varvil,1984; El-Zik & Thaxton, 2000; Savoy, 2005), and values > 60% are widely accepted as adequate for planting seed. However, it is important to note that while the cool germination test is not required by law, it best represents a seed germination potential under less-than-ideal conditions. Still, due to a relatively large variability in CG test values, many in the industry do not believe it provides a reliable measure of planting seed quality. Tolliver et al. (1997) (conducted a cool germination referee project in which two samples were submitted to twenty-seven commercial, state, and independent seed-testing laboratories. The authors concluded that only nine responding laboratories were within the acceptable germination tolerance levels. They suggested that maintaining a constant 18 °C ± 0.5 °C and the germination substrate's type and moisture content were the most critical elements to this test. To this day, the cool germination test is not listed on the seed tag.

Seed testing and regulation play a pivotal role in safeguarding the quality of seed produced and sold within the United States. The requirements for certified seed in terms of quality include both germination rates and viable seed (germination percentage plus hard or dormant seeds) (NC General Statutes, 1963). The certification criteria do not include the percentage of visual mechanical damage (VMD) that remains in the bag after processing. Damaged seeds often evade scrutiny despite their impact, rendering seeds more susceptible upon planting and potentially compromising yields. Damaged seed may not germinate or may germinate but encounter development problems that could lead to adverse effects on plant growth later in the season.

Posting the rates of VMD of a commercially sold seed bag is critical information needed to assist growers in making proper adjustments to their seeding rates.

For North Carolina, the North Carolina Seed Law of 1963 requires the seed tag to have the following requirements: (1) lot identification; (2) origin, if known, if unknown, so stated; (3) kind and variety; (4) name and number per pound of noxious-weed seeds; (5) percentage of germination with month and year of tests; (6) name and address of the person who labeled the seed or who sells offers, or exposes the seed for sale. The percentage of visual mechanical damage (VMD), which refers to seeds that have a damaged seed coat, is not on the seed tag for any state in the US and is only found on the seed tag within the country of Australia Cotton Seed Distributors (CSD), (Personal communication September 6, 2022). At times, a seed can be extremely damaged with an exposed embryo. Despite the strength of cotton seed coats, each seed is susceptible to various damage, stemming from both minor and severe impacts from the harvest and ginning process. The percentage of visual mechanical damage in the bag post-processing is currently absent from the certification criteria. Despite its impact, damaged seeds can drastically and negatively impact germination, leading to compromised yields (Crisp & Unruh-Snyder, 2023).

In recent years, grower concerns specifically pertain to cases where planting conditions were deemed adequate, yet fields did not achieve acceptable stand establishment. A nationwide cotton seed quality survey project was initiated to address these stakeholder concerns in 2019. This project represents a collaborative effort among Extension Cotton Specialists and is supported by Cotton Incorporated, a non-profit organization representing cotton growers in the United States (Maeda et al., 2021). The notable decline in seed quality has been exacerbated by the trend of diminishing seed sizes, increasing seed costs, and the necessity for growers to cultivate larger acreages under suboptimal conditions with a good, uniform stand. From a commercial perspective, the industry widely utilizes germination tests conducted under controlled conditions to determine planting seed quality. Because seeds are living organisms, determining quality and predicting field performance is often challenging, especially since field conditions vary and can be widely unpredictable.

Due to how many factors impact seed development, determining quality related to performance can be challenging. It has long been acknowledged that cotton seeds have a dormancy period in the weeks following harvest (Christidis, 1955; Hsi & Reeder, 1953; Simpson, 1935). This adds another level of logistic complexity for companies needing to deliver a quality product to farmers targeting early planting dates and less-than-ideal conditions. Cotton is a perennial shrub cultivated as an annual crop. Because of this characteristic, the development of fruiting sites (and seed) occurs, exposing the fruit to varying stress sources and levels during a growing season. Studies have shown that seed quality, as determined by seed yield, kernel, seed weight, and oil content, differs based on its position on the plant (Conkerton et al., 1993). Low nighttime temperatures during seed development have also been reported to negatively affect germination, oil, and nitrogen content compared to seeds produced at 20 to 25 °C (Gipson & Joham, 1969).

In addition, field studies have shown that plants emerging early tend to have the highest survival rates. Wanjura et al. (1969) noted that the average survival rate of plants emerging on the 5^{th}, 8^{th}, and 12^{th} day after planting was 87%, 70%, and 30%, respectively. This has significant implications for farmers' ability to establish a uniform stand with high yield. Thus, finding ways to relate seed damage to the field or other lab practices, such as warm or cool germination tests, is critical. For instance, seed size is a coadapted feature of plant life history (Silvertown, 1981). Within the cotton industry, seed size is estimated and expressed as the number of seeds per unit weight (i.e., seeds/lb, seeds/kg). Seed size has long been a topic of interest since it can be associated with overall plant establishment, mortality, and initial seedling vigor (Buckley, 1982; Snider et al., 2014; Westoby et al., 1992). However, it is essential to note that an advantage at the field level may only be realized under certain environmental conditions. In the field, seedlings from large seeds had a higher percentage of emergence and survival than small-seeded seedlings. Also, the daytime temperatures were very high during the field experiment, and even the most favorable watering treatment showed relatively low rates of emergence and survival. This low survival may have reduced the ability to detect a greater advantage of large seeds over small seeds under drier than under better-watered conditions (Leishman & Westoby, 1994). This can be the case with many seed quality characteristics, such as cool germination, which should be considered for decision-making. Also, small seeds can create issues that go well beyond the field boundaries, as can be the case with gins, where small and fragile seeds can contaminate lint and affect spinning performance (Bechere & Hardin IV, 2021). Cotton is a unique crop in that both the seed and the fiber are commercially important.

Similarly, the seed index is measured as the weight in grams of 100 seeds and provides an indirect measure of seed size by a measure of weight. Cotton breeding programs often use this measurement to calculate yield components (Groves & Bourland, 2010). Maeda et al. (2023) recently noted a significant shift in the seed index of Texas's ten most planted cotton varieties between 2008 and 2018. By cross-referencing the USDA Agricultural Marketing Service’s estimated variety planted acreage with the Texas A&M AgriLife Cotton Performance Tests conducted in the Texas High Plains, the authors presented a clear trend toward smaller seeds for the varieties occupying most acres in the State. Approximately 48% of the total Texas cotton acreage in 2008 was represented by varieties with seed index greater than 10.6. In contrast, in

2018, approximately 69% of the acreage was occupied by varieties with seed index ranging from 8.5 to 9.5. No varieties in 2018 had a seed index greater than 10.6.

In addition, the seed oil is essential in evaluating seed health because it has been tied to seed quality as an indicator of yield; with about 21% oil and 23% protein by weight in the seed, cotton is also the fifth-largest oil crop in the world (Liu et al., 2012). Damaged seeds are more prone to moisture uptake and oil degradation during storage, which causes a direct loss of extractable oil and oil refining problems from degradation (Dowd et al., 2018). Moreover, the cotton seed's total oil percent plus protein calorie content would strongly predict seedling vigor across most field conditions, and seed traits' impact on yield would depend on the yield environment. Thus, for a given seed variety, the total oil percent ranging from 12.7% to 22.4% may represent a strong indicator of vigor, regardless of whether variation in seed and seedling characteristics are genotype or environment based. While differences in seedling vigor do not always impact yield in cotton (Liu et al., 2012; Snider et al., 2016), it should be noted that there were other positive aspects associated with early season vigor.

Relating different factors of seed health to their relationships with VMD is a way to determine if reporting VMD can be instrumental as an indicator of seed quality. Since there is currently no industry-standard method for VMD measurement, it is not easy to compare these numbers. Douglas et al., 1965 were the first reference of methodology to determine cotton seed damage. They tested four varieties, using 100 acid-delinted seeds to determine cotton seed damage by a mechanical harvester. The damage grade reported was the following: (0) no visible damage; (1) seed coat scratched or bruised but not broken; (2) seed coat broken slightly but embryonic portions of seed not visible through break; (3) seed coat broken sufficiently so that portions of cotyledons or other embryonic parts were visible through break; (4) large section or all the seed coat removed or nearly so and broken seed. They concluded that as the grade level increased from 2 to 4, the germination rate decreased by at least 50%, and the link between VMD and the germination rate was negatively correlated. Souza et al. (2009) noted that the severity and location of the damage are essential factors, as more damage to the seeds in the regions of the radicle and plumule would reduce germination and vigor. The cotton seed industry would benefit from a standardized methodology and an understanding of how VMD correlates to seed quality, if not for seed production distribution, then for growers.

Materials and Methods

The study was conducted at the North Carolina State University's (NCSU) seed laboratory in Raleigh, North Carolina, in May 2021. Seed samples were collected from all commercially available varieties of cotton seed distributed the same year, which were delinted and treated. Cotton Incorporated provided samples alongside existing information on each sample, along with data collected from the Cotton Extension specialist with key parameters such as stand count at 7 and 10 DAP, seed density, seed index, percent oil, seed size, warm and cool germination rates, and percentages of VMD.

A request for information was sent to each seed company individually, and based on a sample's lot number, the information requested included warm and cool germination, seed size, and VMD from the companies. There was no attempt to determine where and when each company tested seed, though it is understood that most were tested internally and with a third party. Companies contacted were generally responsive to this request, and most, but not all, information requested was received. Information was coded for each company and variety, and they are consistently represented throughout the manuscript.

Samples were gathered from lots spanning 12 states: Alabama, Florida, South Carolina, North Carolina, Texas, Mississippi, Arizona, Arkansas, Virginia, Georgia, Tennessee, and Oklahoma. Alabama and Texas had two locations each, for a total of 14 locations. Seven major cotton seed brands commercializing seeds in the United States were represented. A total of 41 individual varieties (198 samples) were surveyed, including 168 individual seed lots.

The seed was sent to the North Carolina Department of Agriculture (NCDA) for warm and cool germination in April 2021. Tests followed the guidelines described by the Association of Official Seed Analysts Seed Vigor and Testing Handbook (AOSA, 2020). A standard commercial check variety was also shipped from select locations. While the variety was the same, different lots were used.

Companies reported warm germination in January 2021. In addition, the NC State seed laboratory conducted warm germination tests in May of 2021. The 196 samples were composed of fungicide-based treated seeds with seed coloring analyzed for VMD analysis at NC State and were sorted, photographed, and germinated using methodologies adapted from Douglas et al., 1965; Douglas et al., 1967; ARS 1972; and McCarty and Baskin, 1978. The seeds were sorted into four categories: (1) normal, with no visual imperfections; (2) pinhole, a small symmetrical hole in the seed coat; (3) damaged, a crack or abrasion on the surface of the seed that does not reach the interior embryo, and (4) very damaged, exposure of the embryo as shown in Figure 1.

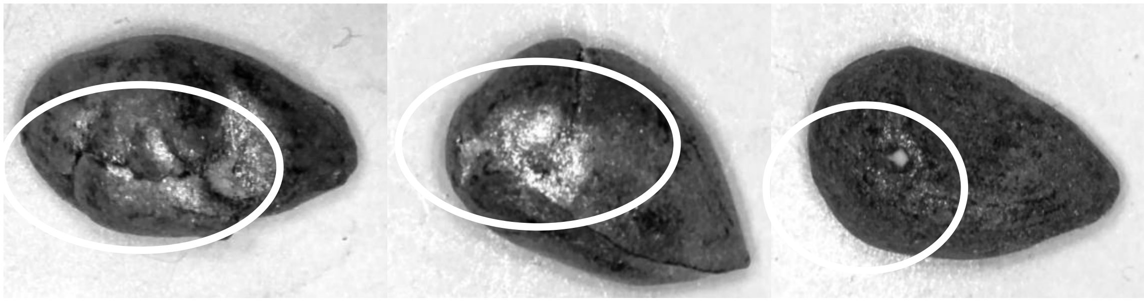

Figure 1. From left to right are examples of visual mechanical damage enclosed within the white circle. The very damaged seed (left) is a misshaped seed with an exposed embryo; the damaged (middle) has a vertical crack; and the pinhole (right) has a center hole within the white circle.

After VMD was calculated, the next step was to germinate the seeds. A typical warm germination test would be germinated as 50 seeds placed on a paper towel, rolled, germinated, and never sorted for VMD seeds. The procedures created for this study were adapted in two ways: the non-damage seeds were placed in the rolled towels and germinated, and the VMD seeds were placed in the germination boxes and given a number for tracking to follow the seed's fate to seedling germination. Those assigned numbers stayed with the seed through the germination process. Four seeds were placed in each germination box, 10.16 cm x 10.16 cm x 3 cm, and the numbers were placed in each quadrant. The boxes were photographed with the seeds before entering the germinator (Figure 2). Two layers of fully saturated germination paper were placed at the bottom of the box, and then two more layers of moistened germination paper covered the seeds. Since this had never been done before, tracking and confirming each germinated seed's seedling performance was critical to reporting the percentage of normal and abnormal seedlings for a warm germination test (AOSA 2020). The germination boxes were then stacked and placed into plastic bags to contain the moisture within them while in the germinator. The boxes held moisture; no additional water was added throughout germination. The seeds were germinated using a standard warm germination technique (AOSA 2020) at 20-30℃ with light for 8 hours during the 30 ℃ period and dark for 16 hours during the 20 ℃ period. On day 10, the seeds were ready to be evaluated for their germination results, and each seedling was measured, and data was recorded for tracking. To ensure consistency and reliability when reporting, VMD testing methods were used based on the developments from Crisp and Unruh-Snyder (2023). On the final day of germination, the images were taken, and each seed was categorized according to its performance and health and given a vigor rating.

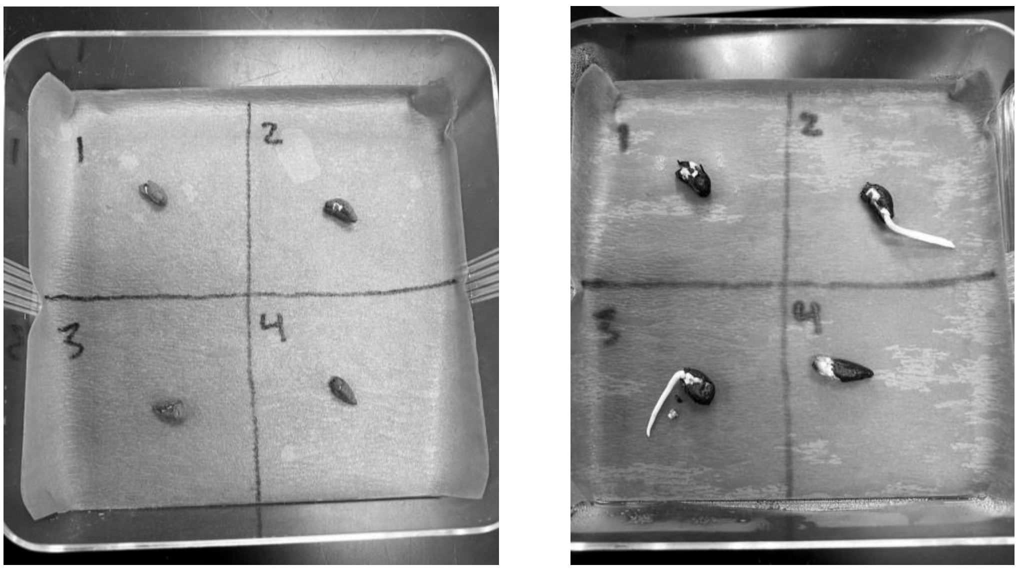

Figure 2. The seed boxes used in the germination were tracked with individual numbers and photographed throughout the germination process. Four damaged seeds are shown on Day 0 (left) and Day 4 (right).

The seed or seedlings were placed in the following categories: strong (greater than 8 cm), weak (8 to 4 cm), abnormal (greater than 4 cm), abnormal (less than 4 cm), dead, or hard seed to categorize the vigor of each seed/seedling. The following formula was created to understand how VMD seed impacts the performance of seedlings. If the seedling was in the category of 4 to 8 cm and was normal, it was assigned a value of 0.75 on a performance scale of 0 to 1. If the seedling was over 8 cm, it was assigned a value of 1. In addition, if the seedlings were abnormal, they would not be counted in the AOSA 2020 procedures; however, this method was adapted to account for the abnormal seedlings surviving in the field. The new procedure was now considered the VMD germination standard.

These adjustments were based on the established strong, weak, and abnormal categories in the ISTA Handbook of Vigour Test Methods (Hampton et al., 1995), each with specific parameters relating to the seedling and its appearance after the germination test. The data collected for the VMD seed included the type of VMD, length of radical, and category of growth (strong, weak, abnormal).

The data was analyzed to produce a percentage of average germination based on the greater than 4cm method from AOSA (2020). The collected data was analyzed and combined to compare percentages gathered from the sets with the warm germination percentages produced by the NCDA, NC State, and the company. For each paper towel roll, the percentage was indicated as strong and weak, describing the number and type of abnormalities.

For the oil and protein, the cotton seed samples were placed in a desiccation chamber and lyophilized for a minimum of 12 hours to remove all moisture. Representative aliquots of cottonseed were taken from each sample (three aliquots; approximately 3.0 g each) and placed in 18-mm-diameter glass nuclear magnetic resonance (NMR) tubes. Samples were packed down to occupy minimal volume and maximize sample homogeneity. Three aliquots per original tube were measured, and the average was considered the average of the three biological replicates. 1H-NMR signals were recorded on a Bruker minispec mq20 NMR instrument (Horn et al., 2011). The pulse-field, time-domain signals obtained for the protein and oil parts of the 1H-NMR spectra were utilized in a chemometric algorithm developed explicitly for cotton seed to calculate the amount of total protein and oil in the seed sample, and they are reported as % by weight. The instrument was calibrated to known standards daily. Graphs were compiled using sample averages, and standard deviations were calculated. Because seed samples were from commercial seed lots, protein data was ignored since it is known to be affected by seed treatment.

The delinted and treated cottonseed samples were counted into 25 seed aliquots and weighed to determine the total mass for the seed index and volume. The determined mass was then multiplied by 4 to determine the seed index in g/100 seeds. A 25mL graduated cylinder was filled with between ~10mL and ~20mL of 80% Isopropanol mixed with 0.1mL of polysorbate 80 to reduce surface tension. Seed aliquots were added to the cylinder, and the volumetric displacement was used to calculate seed density.

In addition, the seed samples from the survey were planted in small plot experiments at select locations across the Southern United States. Stand counts 7 and 10 days after planting (DAP) were collected at each field site. While planting equipment and other factors varied by location, soil and air temperatures were monitored through the first ten days after planting at each location. For reference, soil temperatures at the 10 cm depth ranged from 15.6 to 32.8 °C, and ambient temperatures ranged from 7.2 to 41.7 °C for the first 5 days following planting, indicating that conditions were at a minimum adequate for cotton.

The VMD warm germination percentage and other VMD variable formulas were correlated with other VMD variables for correlation coefficients by "Pearson's Correlation" method, and simple linear regression models expressed the relationship. A significant regression model was selected, having high model R^2 and adjusted R^2 and significant p-values. Stepwise regression determined which VMD variables predicted stand counts for 7 or 10 DAP %. Data were analyzed using JMP Pro 17.0 for all statistical analysis at a p-value of 0.05.

Results and Discussion

The VMD reported percentage from NC State ranged from 5 to 27% among all varieties (Table 1), while the VMD ratings provided by the companies ranged from 3 to 10%. In total, 196 samples were reviewed for VMD% using the NC State VMD methodology. The vigor ratings given to the tested seed population (15,898 seeds over a total of 55 varieties) were shown in Table 2, as well as the distribution of damaged categories and the results of the seeds/seedlings in each. The total percentage of non-damaged seeds was 87%, where they had 51% in the strong category (<8 cm). The total percentage of non-damaged seeds was 87%, where they had 51% in the strong category (<8 cm). The pinhole was 1.58% of the population, with 23.9% of the strong population (Table 2). The damage type was 3.35% of the population, and 13.32% of its germinated seeds were in the strong category. At the same time, very damage represented 7.89% of the population being the second highest type of seed present in the samples with the lowest rate of seedlings in the strong category at 7.89%.

The seeds were germinated and tracked individually based on their category. The VMD warm germination calculation was based on the categories and was scored as normal, weak, or abnormal. These standards are based on the expansion of the standard germination test. The seedlings obtained from the standard germination test results are further classified (AOSA, 2023). Expanding on these classifications, the North Carolina State University seed lab created the measurement categories strong (> 8 cm), weak (4 to 8 cm), weak (< 4 cm), abnormal > 4 cm, abnormal < 4 cm, dead, and hard seed. Based on this table, the visually most impaired seeds, the very damaged, were 7.89% strong and had little to no chance of surviving. For those that survived, a potential explanation for this is that the location of the damage along the seed coat could have differing impacts due to the physiology of the seed.

Table 1. The mean and standard deviation of 196 samples were reviewed for visual and mechanical damage % (VMD), and the NC State VMD methodology was compared to the company's VMD.

Mean (Standard Deviation) with Two or More Samples and Company Data Provided				
Variety Number (# of Samples)	**Very Damage + Damage + Pinhole %**	**Very Damage + Damage %**	**Very Damage %**	**COMPANY VMD %**
3 (2)	7.8 (4.0)	7.0 (4.2)	4.0 (1.4)	5.0 (0.0)
6 (5)	8.8 (2.4)	7.4 (1.8)	4.4 (1.2)	3.5 (0.7)

Table 1. Continued

7 (7)	9.2 (3.3)	7.5 (1.9)	4.4 (1.7)	3.2 (1.7)
9 (6)	9.3 (3.6)	8.1 (3.3)	5.3 (2.9)	5.5 (2.4)
10 (2)	9.5 (1.1)	8.0 (1.4)	4.1 (0.2)	5.7 (1.1)
12 (2)	9.8 (1.2)	9.0 (1.4)	7.5 (0.7)	6.5 (0.0)
17 (14)	10.8 (2.3)	9.5 (1.8)	7.6 (1.3)	7.6 (0.8)
21 (22)	11.9 (3.9)	10.3 (3.1)	7.0 (2.9)	6.8 (3.3)
23 (8)	12.0 (2.2)	11.4 (2.3)	9.2 (1.8)	7.9 (1.5)
24 (3)	12.3 (1.8)	10.6 (2.9)	7.8 (1.8)	6.3 (1.5)
25 (3)	12.3 (3.2)	10.0 (1.7)	7.6 (2.3)	8.0 (2.6)
26 (4)	12.5 (3.6)	9.5 (1.7)	5.7 (1.8)	6.3 (1.5)
27 (14)	12.6 (3.3)	10.7 (2.7)	8.3 (2.5)	7.4 (2.1)
28 (8)	12.6 (2.2)	11.3 (2.0)	6.9 (1.7)	5.8 (1.2)
29 (4)	13.2 (4.6)	12.3 (2.2)	8.0. (2.2)	6.5 (1.7)
31 (41)	14.0 (3.7)	11.6 (2.9)	7.1 (2.0)	5.9 (2.2)
32 (6)	14.1 (1.7)	11.5 (1.9)	7.5 (1.9)	5.2 (1.6)
34 (9)	17.5 (4.8)	15.2 (3.5)	11.0 (2.1)	7.7 (1.2)
35 (2)	19.2 (8.7)	17.5 (7.8)	15.0 (5.2)	13.5 (5.5)
36 (4)	19.3 (3.5)	18.4 (3.7)	13.8 (1.4)	12.6 (2.8)
38 (2)	20.2 (0.7)	18.0 (0.0)	12.4 (3.3)	12.0 (2.8)
39 (6) (Total 174)	21.5 (3.2)	20.2 (4.1)	18.0 (4.2)	16.3 (3.9)
Mean with One Sample and Company Data Provided				
4	8.7	8.0	7.0	7.0
8	9.3	8.0	6.0	6.0
11	9.7	8.0	7.0	7.5
15	10.7	10.0	9.3	7.0
18	11.3	11.0	8.3	8.0
19	11.7	11.0	8.0	7.0
20	11.7	11.0	7.0	7.0
22	12.0	10.0	7.7	7.0
30	13.7	13.0	10.0	11.0
33	14.7	13.0	7.0	6.0
37	19.3	19.0	15.0	8.0
40	21.7	20.0	14.3	7.0
41 (Total 13)	26.7	26.0	19.0	19.0
Means (Standard Deviation) No Company Data Reported				
1 (2)	5.3 (1.4)	5.0 (1.4)	3.5 (0.7)	n/a
2	7.7	6.0	4.0	n/a
5	8.7	7.0	6.0	n/a
13	10.0	9.0	8.0	n/a
14 (2)	10.7 (1.9)	7.5 (0.7)	4.5 (0.7)	n/a
16 (2) (Total 9)	10.7 (1.4)	9.5 (0.7)	6.4 (0.5)	n/a

Table 2. Vigor ratings for a population of 15,898 seeds were divided into four categories, and the vigor outcome of each seed to the seedling of very damaged, damaged, pinhole, and normal.

		Percentages						
Type	**Proportion**	**Strong (< 8 cm)**	**Weak (4 to 8 cm)**	**Less < 4 cm**	**Abnormal > 4 cm**	**Abnormal < 4 cm**	**Dead seeds**	**Hard seeds**
Very Damage	7.89	7.89	26.16	12.92	4.07	11.56	34.61	0.08
Damage	3.35	13.32	41.46	16.70	1.13	1.13	22.14	0.00
Pinhole	1.58	23.90	45.82	13.94	3.19	3.19	9.56	0.40
Non-Damaged	87.18	51.00	33.22	3.19	4.20	4.20	6.05	0.66

The relationship between the company-reported VMD and the VMD calculation of very damaged (VD) plus damaged (D) plus pinhole (P) or (VD + D + P) was significant and had a positive correlation of 0.5619 (Table 3). Without knowing the exact methodology of each company, the two had a strong relationship. This relationship was stronger when looking more closely at the measurement of the VD % seed category alone. Table 3 also displayed the significant positive correlation of 0.8556 between itself and the company's reported data. Based on the increase in positive correlation, the techniques used by the companies to analyze the percent VMD may only include just the VD percentage since it was more closely correlated than the other two VMD % related to damage and pinhole. In Figure 3, the R^2 values were all significant at a p-value of <.0001 and were the following: VD + D + P % was 0.32, VD + D % was 0.45, and VD % was 0.73 with the respective F-values: 83.07, 149.23, and 491.72. Thus, VD % was the closest to the reported company VMD percentages.

Table 3. Visual mechanical damage (VMD) variables correlated to the percentages of the company VMD, company and NC State (NCSU) warm germination, company cool germination, stand counts at 7 and 10 DAP, seed oil, and VMD very damaged (VD), damaged (D) and pinhole (P) seeds. In addition, seeds per kg were correlated with the VMD warm germination percentage.

Variable (Percentages)	by Variable	Correlation	Count	Significant Prob
Company VMD	Oil %	-0.27	178	0.0002
NCSU VMD, VD	Oil %	-0.26	189	0.0002
Company Warm Germination	Oil %	0.17	180	0.0162

Table 3. Continued

NCSU Warm Germination	Oil %	0.36	37	0.0245
VMD Warm Germination	Oil %	0.39	37	0.0161
Index	Oil %	0.42	191	<.0001
VMD Warm Germination	Index	0.47	37	0.0032
VMD Warm Germination	Stand Count at 10 DAP %	0.66	37	<.0001
VMD Warm Germination	Stand Count at 7 DAP %	0.58	24	0.0027
Company Warm Germination	Seeds per kg	-0.27	177	0.0002
NCSU Warm Germination	Seeds per kg	-0.49	33	0.0031
VMD Warm Germination	Seeds per kg	-0.52	33	0.0018
VMD, VD+D +P	Seeds per kg	0.16	176	0.0265
Company VMD	VMD, VD %	0.85	182	<.0001
Company VMD	VMD, VD + D %	0.67	182	<.0001
Company VMD	VMD, VD + D + P%	0.56	182	<.0001
VMD Warm Germination	NCSU Warm Germination%	0.98	37	<.0001
VMD Warm Germination	Company Cool Germination %	0.5263	36	0.0010

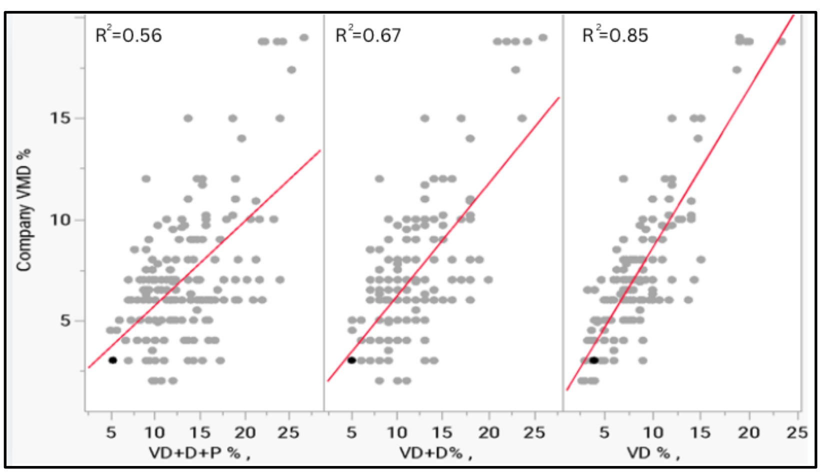

Figure 4. The visual mechanical damage (VMD) correlations were significant at a p-value of =<.0001. They were the following for the R^2 values: very damaged (VD) plus damaged (D) plus pinhole (P) seeds: 0.32; VD + D seeds: 0.45; and VD seeds: 0.73 with the respective F-values: 83.07, 149.23, and 491.72 Thus, VD % was the closest to the reported company VMD percentages.

VMD was checked by having a control (the same variety and lot) sent to all states to see if the very damaged percentages were different, but they were not. The range for very damaged seeds was 6% to 10%.

The average seed size reported by seed companies for the samples surveyed was 12,137 seeds/kg. Most samples (53%) were reported to be between 12,000 and 13,999 seeds/kg, with all samples ranging from 7,000 to 16,000 seeds/kg (Figure 5). In addition, the samples included in the survey ranged from a seed index of 5.5 to 12, with the biggest class represented by samples with a seed index of 8. The VMD total percentage calculated by NC State had a positive correlation (0.1672) to seeds/kg with a p-value of 0.0265 (Table 3). Further investigation was conducted and reported to determine if seed size influenced visual mechanical damage (see Table 4). The results varied in such that overall total VMD were not different between small and medium or medium to large, very damage % or very damage plus damage % was not significant between seed sizes, and pinhole showed to be more prominent in smaller seeds than medium or large. Table 6 displays the variability of the VMD % found within seed size and lots, further suggesting that seed size may not be linked to an increase or decrease in damage.

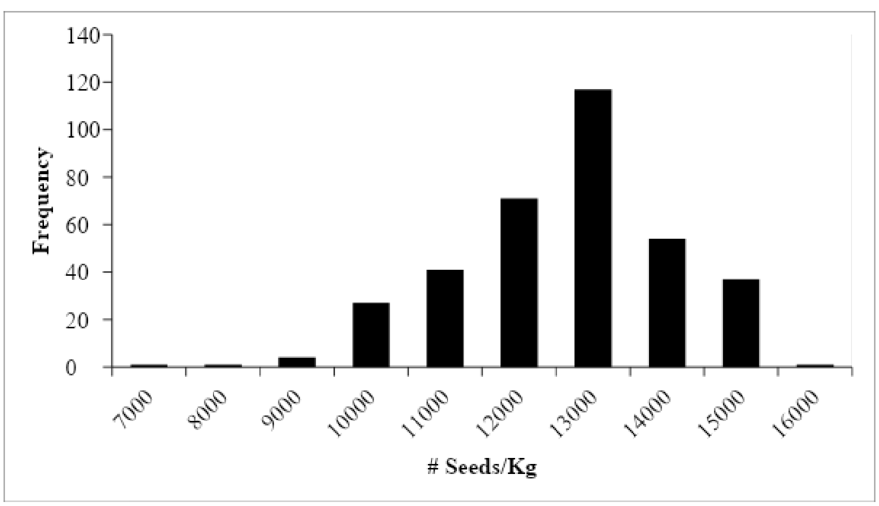

Figure 5. Seed size frequency distribution as reported by private seed companies (*n* = 354) with an average of 12,137 seeds/kg.

Table 4. Influence of Seed Size on VMD. Levels of visual mechanical damage (VMD) based on size.

Seeds per kg	Total Visual Mechanical Damage %	Very Damaged %	Damage %	Very Damaged + Damaged %	Pinhole %
Level	Mean	Mean	Mean	Mean	Mean
Small: 13,500-16,000	14.4 A	8.4 A	3.7A	11.8 A	2.5 A
Medium: 11,400-13,400	13.3 AB	8.3 A	3.5 AB	11.6 A	1.7 B
Large: 9,004-11,220	12.3 B	8.2 A	2.9 A	11.3 A	1.0 C

Note: The seeds per kg not connected by the same letter are significantly different at a p-value of 0.05. Seed size was significant when looking at total visual mechanical damage across all the samples. The significance relates to the small seeds (13,500 to 16,000 seeds/kg) and large seeds (9,004 to 11,229 seeds/kg-^{1}).

To understand the relationships between the VMD% and warm germination rates, correlation tests were performed and reported in Table 3, where the six VMD-related and warm germination percentage variables correlated to oil %, company cool germination %, NC State warm germination %, VMD (Very Damaged [VD]) %, VMD with VD + (Damaged [D]) %, VMD with VD, D, + (Pinhole [P]) %, seeds per kg, stand count at 7 and 10 DAP % and seed index. In addition, the seed index was correlated to the seed oil index to compare with other variables measured.

The correlation between VMD warm germination and company cool germination averaged 0.5263, with a lower range of 0.2391 and a higher range of 0.7288 (Table 3). This helped to explain how closely VMD warm germination was related to cool germination or seed vigor. The company's warm germination percentage and the company's cool germination percentage were not significant when correlated. Thus, the VMD warm germination calculation was more closely related to field performance or stand counts, which were also used in developing the stepwise regressions in Table 9.

The correlation between seed size and warm germination percentage for the company, NC State's warm germination percentage, and the VMD warm germination percentage was negative for all three, with the highest negative correlation reported at -0.5325 for the VMD warm germination percentage. Based on the significance, it can be concluded that larger seeds do not equal healthier seeds. As seed size decreases, that may be less of a concern than presented biologically due to smaller seeds performing better overall (Table 3).

The relationship between visual mechanical damage (VMD) warm germination percentage and stand counts 10 DAP % had a positive correlation of 0.6662 and a p-value of <.0001 (Table 3). The higher the VMD warm germination, the higher the stand count of 10 DAP in the field, as seen in Figure 5.

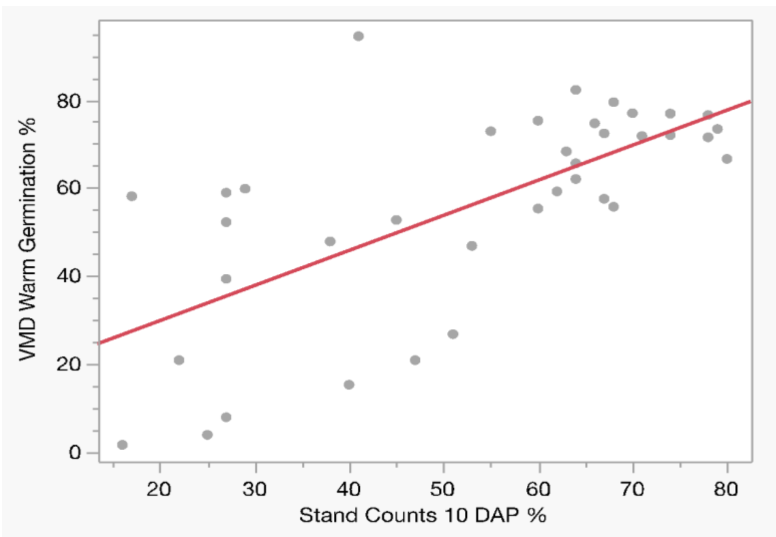

Figure 6. The relationship between visual mechanical damage (VMD) warm germination percentage and stand counts 10 DAP % had a positive correlation of 0.6662 with a p-value of <.0001. The higher the VMD warm germination, the higher the stand count of 10 DAP in the field.

As represented in Table 3, there was a negative correlation between seed oil percentage and VMD percentage calculated by the company (-0.2715) and NC State's VMD with VD % (-0.2695). Both were significant with a p-value = 0.0002, meaning there would be a lower oil percentage with a higher percentage of damage. However, a positive relationship was indicated between the company's warm germination (0.179), NC State's warm germination (0.3693), and the VMD warm germination (0.3929) percentages and the oil percentage, suggesting that the higher the oil content, the more likely the seeds would germinate. The highest correlation was between the VMD warm germination percentage and oil percentage, which ranged from a lower correlation value of 0.0789 to an upper 95% value of 0.6360. The mean percentage for the population of seeds evaluated for the oil was 18.58 % with a standard deviation of 2.39 %, and the VMD warm germination percentage mean was 56.03% with a standard deviation of 23.72 %. The covariance was 27.2945, and the count was 37 samples. Because the sample size was 37, this was a limitation of the study and thus represented an opportunity to collect additional data to improve the relationship.

Table 6 displays the significant relationship, indicated by an ANOVA, between the VMD warm germination percentage and oil percentage in the seeds tested based on this study and the results provided in the company data. For NCSU-generated data, there was an increase in warm germination per oil unit of 3.1832, while company-reported data shows an increase per unit of 0.2472. The linear fit was VMD Warm Germination % = -4.540411 + 3.1832898* Oil %, which had an R^2 value of 0.154 and a p-value of 0.0161, where the lower the oil percentage, the lower the VMD warm germination, and the higher the oil percentage, the higher the VMD warm germination percentage has displayed in Figure 7.

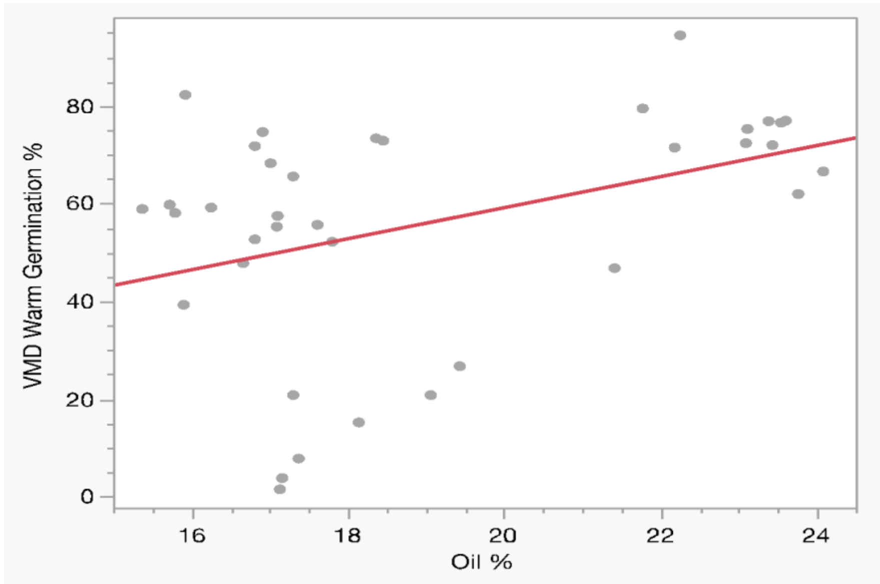

Figure 7. The average correlation was 0.3929 with an R^2 value of 0.154 and a p-value of 0.0161, where the lower the oil %, the lower the VMD warm germination, and the higher the oil percentage, the higher the VMD warm germination percentage. Adjusted NCSU VMD was correlated at a higher value than the standard warm germination test.

Table 5. A summary table of the variables included was used to create stepwise regression equations with R2 values over 0.40000 values to predict stand counts at 7 or 10 DAP percentages.

Description of Variables	Variable Symbol	Unit	Stand Count DAP %	MSE	R^2	Adjusted R^2	Included Variables
Visual Mechanical Damage (VMD) Warm Germination	A	%	7	9.302496	0.62886	0.585197	AC
NCSU Warm Germination	B	%	10	15.54871	0.414218	0.375166	AC
Seed Size	C	Seeds per lb	7	9.424844	0.542008	0.498390	AG
VMD, Very Damaged (VD) Seeds	D	%	10	13.68977	0.549329	0.522819	AG
VMD VD + Damaged (D) Seeds	E	%	7	8.929092	0.678169	0.617825	ACE
VMD VD + D + Pinhole (P) Seeds	F	%	10	15.75468	0.418643	0.358502	ACE
Oil	G	%	10	14.02046	0.541196	0.499487	BDF
Normal Seed	H	%	10	13.43166	0.591682	0.540643	AGHF

Note: See Appendix C-F for further information.

The summary Table 5 displays the variables included to create stepwise regression equations with R^2 values over 0.40000 to predict stand counts at 7 or 10 DAP percentages. When looking at seed size as an indicator of stand establishment, an R^2 value for 7 DAP of 0.62886 indicated a significant variation in the percentage of seeds germinating at 7 DAP between VMD germination and seed size, as seen in Table A.

The variables VMD warm germination % and oil % were significant predictors of stand counts 10 days after planting (DAP). An increase in either variable will increase stand counts indicated by the R^2 of 0.522819.

The results shown in Table 5 show that VMD warm germination and seeds per pound were the more significant predictors of stand counts at 7 DAP and 10 DAP. The VMD warm germination percentage had a p-value of 0.00075 and an R-square value of 0.418643. The seeds per lb. had a p-value of 0.00180 and an R-square value of 0.236. The VMD percentage for very 79 damaged (VD) plus damage (D) seeds was not a significant predictor of the percentage of stand counts at 7 DAP and 10 DAP.

In Table 5, the comparison between VMD and VD + damaged (D) seeds, the equation for the model is: Logworth 10 DAP% = 41.444526 - 0.323136 * VMD, VD + D%—0.002531 * Seeds per lb + 0.4945033 * VMD Warm Germination %. This equation can predict the percentage of stand counts at 7 DAP and 10 DAP for the independent variable's given set of values.

In Table 5, the coefficients for the VMD percentage for VD seeds and NC State's warm germination percentage were both significant (p-value < 0.05). The coefficient for the VMD percentage for very damaged (VD) plus damaged (D) plus pinhole (P) seeds was not significant (p-value > 0.05). With an R^2 of 0.499487, the model can be used as a predictor for stand counts at 10 DAP; however, it would be best to continue to improve this model over time with multiple locations and sample numbers.

In Table 5, the variables VMD, Warm Germination %, and Oil % all significantly affected the percentage of non-damaged seeds, with an R-squared value of 0.591682. However, the VMD, VD + D + P % variable, and non-damaged % were not statistically significant predictors of 10 DAP stand counts.

Company Warm Germination % to Stand Counts 10 DAP% probability was not significant with a p-value of 0.3148 compared to the VMD warm germination percentage of <0.0001, and compared to the NCDA warm germination percentage, the R^2 value was only 0.08 compared to the VMD warm germination which had an R^2 value of 0.44 (Table 6). Company warm germination % to stand to count at 10 DAP % had an R^2 value of 0.0106. NCDA warm germination % to stand counts at 10 DAP % had an R^2 value of 0.0864. The adjusted VMD Germination % to stand to count at 10 DAP % had an R^2 value of 0.4438. Based on Table 11, the data suggests that the adjusted VMD Germination % was the best predictor of stand counts.

Table 6. Company Warm Germination % to Stand Counts 10 DAP% probability was not significant, has a p-value of 0.3148 compared to the VMD warm germination percentage of <0.0001, and compared to the NCDA warm germination percentage, the R^2 value was only 0.08 compared to the VMD warm germination which had an R^2 value of 0.44.

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	1	8993.844	8993.84	27.9370
Error	35	11267.657	321.93	Prob > F
C. Total	36	20261.501		<.0001*
Intercept	13.696726	8.535596	1.60	0.1176
Stand Count 10 DAP %	0.7975668	0.150896	5.29	<.0001*

Table 7. The summary of fit for the company warm germination % to stand to count 10 DAP% probability was insignificant and had a p-value of 0.3148 compared to the VMD warm germination percentage of <0.0001. Compared to the NCDA warm germination percentage, the R^2 value was only 0.08 compared to the VMD warm germination, which had an R^2 value of 0.44.

	Company Warm Germination % to Stand Count at 10 DAP %	**NCDA Warm Germination % to Stand Count at 10 DAP %**	**VMD Germination % to Stand Count at 10 DAP %**
RSquare	0.010635	0.086493	0.443888
RSquare Adj	0.000221	0.077266	0.427999
Root Mean Square Error	3.724407	6.627241	17.94249
Mean of Response	91.58969	88.10891	56.03243
Observations	97	101	37

Conclusions

The objective of the current study was to assess variations between market-available cotton seed varieties. It found correlations between different seed factors, focusing on visual mechanical damage, seed size, seed oil, and warm germination rates. It also correlates information provided by cotton seed distributing companies to the measurements found in this study about seed quality. Visual mechanical damage was a significant indicator of seed quality related to oil, seed size, and germination.

Visual mechanical damage is a factor to be considered when measuring seed health and vigor for planting. It impacts seed health and is detrimental to growth and development. Even with the variation across varieties, it remains present and impactful as an indicator of seed quality. A clearer understanding of seed quality indicators can lessen the threat of poorer stands when there is a high risk of early season abiotic and biotic pressure on the seeds, including pests, disease, cool temperatures, and drought, all typical early growing season factors in cotton-producing regions.

Due to differences in total VMD percentages across varieties reported when comparing the results of this study to the data provided by the seed companies, concerns should be raised about the accurate representation of seed quality indicators. This concern is based on testing standards that indicate a higher negative impact when more than just the significantly damaged class of seeds is present.

Also, as mentioned, two of the most common germination tests for cotton are the standard warm germination test (20/30 °C with 8 hours of light) and the cool germination test (18 °C with 24 hours of dark). While the current industry standard, these tests establish the maximum plant-producing ability of a seed lot (AOSA, 2020) but do not always approximate or predict potential plant productivity in a field setting. The results shown with the VMD warm germination relate more closely to predicting stand counts than the warm or cool germination and were not found to be significant in the correlations or the stepwise regressions. Thus, the VMD warm germination calculation is a valuable tool. The measurements used to determine seed health were the extent of visual mechanical damage present in the sample, the germination rate, and the vigor of the seedlings produced. These measurements provide valuable insights into the field potential of seed lots beyond just warm and cool germination rates. Given the many factors impacting cotton production and crop success, studying VMD in cotton seeds offers an additional and advantageous tool for seed health evaluation.

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Chapter 4

The Development of the Visual Mechanical Damage Classification Test (VMDCT) to Determine the Impact on Cotton Seed Germination and Vigor

Abstract

Mechanical damage significantly impacts cotton (*Gossypium hirsutum*) seed quality and decreases germination and seedling establishment due to injury. While previous studies have acknowledged the adverse effects of visual mechanical damage (VMD) on seed viability, the specific impact of damage location and severity remains understudied. This research aimed to assess the effects of mechanical damage on cotton seeds relative to damage location and severity and its impact on warm and cool germination rates over 12 days. A total of 468 seeds were photographed and categorized based on overall condition and damage location using a Visual Mechanical Damage Classification Test (VMDCT) created at NC State. The VMDCT included nine categories, all relating injury location to the anatomy of the seed coat. Germination experiments were conducted under cool and warm conditions, with germination progress monitored on days 4, 7, and 12. The seedlings were assessed based on AOSA vigor scale criteria. Results revealed significant differences in germination rates and seedling vigor among the nine categories, with seeds categorized as very damaged exhibiting notably reduced germination and vigor. Damage location significantly impacted seedling performance. These findings show the importance of assessing mechanical damage in cotton seeds and its implications for seedling vigor and crop productivity. Implementing seed processing practices to minimize damage and enhance seed quality could improve cotton seedling establishment and yield increase.

Introduction

This study documented the locations of visual mechanical damage (VMD) on the cotton (*Gossypium hiristum*) seed coat, its specific impact on seedling performance, and the seedling vigor monitored over a 12-day at 4, 7, and 12 days. There is currently no industry-standard method for VMD, and specific damage patterns have yet to be classified, making it difficult to compare the data gathered in this study.

The ability of a seed to grow is determined by its overall health and quality as it is processed from plant to ground. Researchers have measured a seed's health and ability to grow in various ways, including germination testing (AOSA 2023), vigor ratings (AOSA 2023), x-ray examinations (Elias et al., 2009), oil and amino acid profiles, variety trials (Elias et al., 2009), and overall genetics (Elias et al., 2009). These methods of measuring seed health provide meaningful data but could be expanded on and improved.

The visual mechanical damage to cotton seeds adversely affects the seed and its potential to yield healthy seedlings compared to undamaged seeds. However, it is noteworthy that not all damaged seeds perform poorly, even under severe damage conditions. This variance in performance may be attributed to the inherent morphology, how it impacts the physiology of the cotton seed, and the specific location of the damage on the individual seed.

A previous study developing categories related to visual mechanical damage done by Douglas et al.,1965 and Douglas et al., 1967, tested four varieties using 100 acid-delinted seeds to determine cotton seed damage by a mechanical harvester. The damage grade reported was the following: (0) no visible damage; (1) seed coat scratched or bruised but not broken; (2) seed coat broken slightly by embryonic portions of seed not visible through break; (3) seed coat broken sufficiently so that portions of cotyledons or other embryonic parts were visible through break; (4)

large section or all the seed coat removed or nearly so and broken seed; however no mention was made of the impact of cuts or abrasions at specific locations on the seed coat. Douglas et al., 1965, concluded that as the grade level increased from 2 to 4, the germination rate decreased by at least 50%, and the link between VMD and the germination rate was negatively correlated. They also reported that the germination rate was negatively correlated, and that germination was reduced if the damaged seed was germinated at 20°C (cool test) compared to 30°C (warm test), indicating that damage was accentuated under non-ideal growing temperatures. The damaged seeds were less likely to germinate successfully and correlated to poor seedling vigor, whereas germinating without damage often produced healthier and more robust seedlings. In addition, the physical damage made the seed more prone to infections by pathogens Douglas et al. (1965, 1967). Therefore, the more damage a seed has, the less likely it is to survive to maturity and perform at the same level as a plant growing from a normal, non-damaged seed (Douglas et al., 1965; Douglas et al., 1967; ARS, 1972; McCarty & Baskin, 1978).

In Brazil, Souza et al. (2009) noted that the damage's severity and location were also important factors but did not provide further details on damage type patterns. They only indicated that more damage to the seeds in the regions of the radical and epicotyl would reduce germination and vigor. In addition, Crisp and Unruh-Snyder (2023) reported similar observations as Souza et al. (2009), with the three main damage categories, very damaged, damaged, and pinhole, for data conducted in controlled germination experiments and the field.

It is important to note that the complexity of cotton seed lies in the composition of the embryo, which must remain intact for seed quality to be preserved. A typical ginned or cleaned cotton seed has an ovoid shape and is pointed at one end, ranging from 7-12 mm long (Figure 1) (Özarslan, 2002). The seed consists of two layers of skin: the outer bark of the seed is woody and hard, and the inner bark is soft and membranous (Sanjarbek, 2023). When the seed is cut crosswise, many small dark spots (gossypol glands) and oil glands are visible (see Figure 1). This oil consists of nutrients used in the growth and development of the embryo (Sanjarbek, 2023). When the outer seed layer is cracked or missing, the oils and nutrients within these layers can be lost and impaired, preventing proper growth and development.

The embryo of a mature cotton seed includes the radicle, hypocotyl, epicotyl, and two cotyledons, which provide the energy for germination and early growth and appear smooth with a shiny surface (see Figure 1). The outer epidermis of the seed coat (or testa) contains the lint fibers. The cotton seed also has a longitudinal ridge or vertical seam called the raphe that runs along the length of the seed, typically from the chalaza cap at the rounded end to the micropyle at the pointed end, the attachment point (see Figure 1). This seam, often called the “suture,” is a natural line where the seed coat splits during germination. This feature is crucial during the imbibition process when water enters through the chalaza cap and moves towards the micropyle, softening the seed coat and creating a moisture reserve for the embryo, where water absorption causes the seed to swell and eventually rupture along the seam, enabling the embryonic axis to elongate when the radicle emerges and becomes the seedling root (Leahy, 1948). Water entering the root hydrates the hypocotyl, which elongates, pushing the cotyledons through the soil. The hypocotyl forms a “hook” as it penetrates the soil. This hook is the first tissue to emerge from the soil (Leahy, 1948).

The cotyledons contain energy for seedling expansion and become photosynthetic immediately upon emergence when in contact with light. The fact that the seed has no plumule delays the seedling display of true leaves until the apical meristem can develop lead primordia. Thus, the seedling stage is long and vulnerable in the crop’s development (Leahy, 1948).

Successful germination and seedling growth depend on these initial physiological and biochemical processes, and any disruption can hinder or prevent the processes (Maeda et al., 2021).

Besides visual mechanical damage hindering seed quality, other factors such as its genetic composition, environmental conditions where the seed was grown, and storage techniques from the plant to when it is planted back into the ground can impact germination (Pinnamaneni et al., 2021). Notably, not all damaged seeds perform poorly, even under severe damage conditions, which can be attributed to the cut's depth and location on the seed (Crisp & Unruh-Snyder, 2023). It is still being investigated, but this could be influenced by seed size, percent oil, and protein (Snider et al., 2016). Thus, these categories and the seed's germination and seedling vigor can help develop key correlations to the components that most critically define a seed's health and vigor for a damaged seed.

Traditionally, vigor testing has relied on cool and warm germination assessments guided by standards outlined in AOSA protocols (2023). However, the impact of VMD on cotton seed germination and vigor has yet to be adequately addressed within the context of cotton seed quality assessment. This research aimed to understand each damaged location's impact on seed vigor and performance.

Figure 1. The anatomy of a cotton (*Gossypium hirsutum*) seed and embryo (Maeda et al., 2021).

Visual mechanical damage (VMD) typically occurs during the harvesting and ginning processes, potentially posing a threat to seed quality. The focus on mass production and removal of cotton from the seed decreases the chance of healthy seeds surviving the ginning process to get the planting (Whitelock et al., 2019). The study also reviewed the patterns of cotton seed damage, relating them to how the damage was caused to the seed. It stated that seed damage consisting of slits and cracks parallel to the minor axis of the seed has been characterized as conveying damage. In contrast, slits and cuts parallel to the major axis of the seed have been defined as damage due to the gin stand (Whitelock et al., 2019). Knowing the point at which a seed was damaged is a good way to determine where adjustments can be made to decrease the rate of visual mechanical damage when ginning cotton. A solution to help mass ginners prevent such damage is to run standard tests on all machines so that the rate of damage can be managed and decreased.

Studies have demonstrated that seeds affected by VMD exhibit decreased germination rates (Douglas et al., 1965), and if they do germinate, they display reduced vigor. These findings have been observed in controlled germination experiments and field conditions. The goal of the project is to create an effective method to determine if the morphology of the seed, as it relates to the presence of damage, impacts the quality of the seedling and, thus, the plant that germinates from a damaged seedling.

Materials and Methods

The study was conducted in the North Carolina State University laboratory in Raleigh, NC, for seed testing. This research aimed to understand each damage location's impact on seed vigor and performance. A total of 468 seeds from a commercially available treated seed variety were categorized as very damaged, damaged, pinhole, and normal and then categorized using the Visual Mechanical Damage Classification Test (VMDCT) created by North Carolina State University before being scanned into a high-quality TIFF image file. The seed categories were based on the anatomy of the cotton seed in Figure 1. There were nine VMDCT categories created based on the damage locations found on the cotton seed coat as follows (Figure 2): normal (N), solid smooth seed coat with no lesions or abnormal marks or indentations; radicle (R) injury to the radicle tip of the seed typically has an opening into the interior of the seed coat; radicle vertical (RV), symmetrical split running vertically along the seed coat opening towards the radical narrow end of the seed; vertical (V) symmetrical split/crack along the entire length of the seed from chalaza end to radical end; radical horizontal (RH) crack/split/lesion on the radical half of the seed coat, either superficial or through to the interior of the seed coat; epicotyl horizontal (EH) crack/lesion directly in the center of the seed coat between the chalaza and radical halves either superficial or through to the interior of the seed coat; chalaza horizontal (CH) crack/lesion on the chalaza half of the seed coat either superficial or through to the interior of the seed coat; chalaza (C) tip of the seed is typically an opening into the interior of the seed coat; chalaza vertical (CV) symmetrical split running vertically along the seed coat, opening towards the chalaza wider end of the seed.

After being scanned, they were stored separately in individual wells of named plates, ensuring tracking for their lifecycle throughout germination. The seeds were split into two sets of 234 seeds so that each damaged location was represented in the cool and warm germination test. In addition, the seeds were individually scanned on two sides to visually represent a sample of any damage or abnormalities on the seed coat. The image analysis system used was the WinSEEDLE 2003b Image Analysis System (Regent Instruments, Inc., Quebec, Canada), specifically designed for seed morphology and analysis.

Each seed was housed in a germination box individually for the germination tests. The germination boxes used were 4 x 4in (10.16 by 10.16 cm). The germination paper was cut to this size, and four sheets of paper were layered in each box using 7.31 mL of water per box. The formula paper weight (g) x 2.15 (mL) was used to calculate the water needed for germination, and distilled water was placed into each box with a micropipette for the cool and warm germination set at 18℃ and 30℃ degrees, respectively. The germination boxes were read, measured, and categorized based on length on days 4, 7, and 12. During each reading, individual images were taken to compare four different images of each seed/seedling at the end of the 12-day growth period. Once all the seedlings were measured and photographed, they were put into further subcategories based on overall performance and vigor. Those categories for performance based on germination were as follows: dead, firm, gray/decayed, short (< 4cm), weak (between 4 and 8 cm), and strong (< 8 cm). The vigor scale is determined by the seedlings' overall appearance and health as they progress through the germination window of 12 days. The vigor rating is then given based on these descriptions and the length of the seedlings recorded in the germination category. The Association of Official Seed Analysts (AOSA) procedures were followed for each germination test and vigor rating.

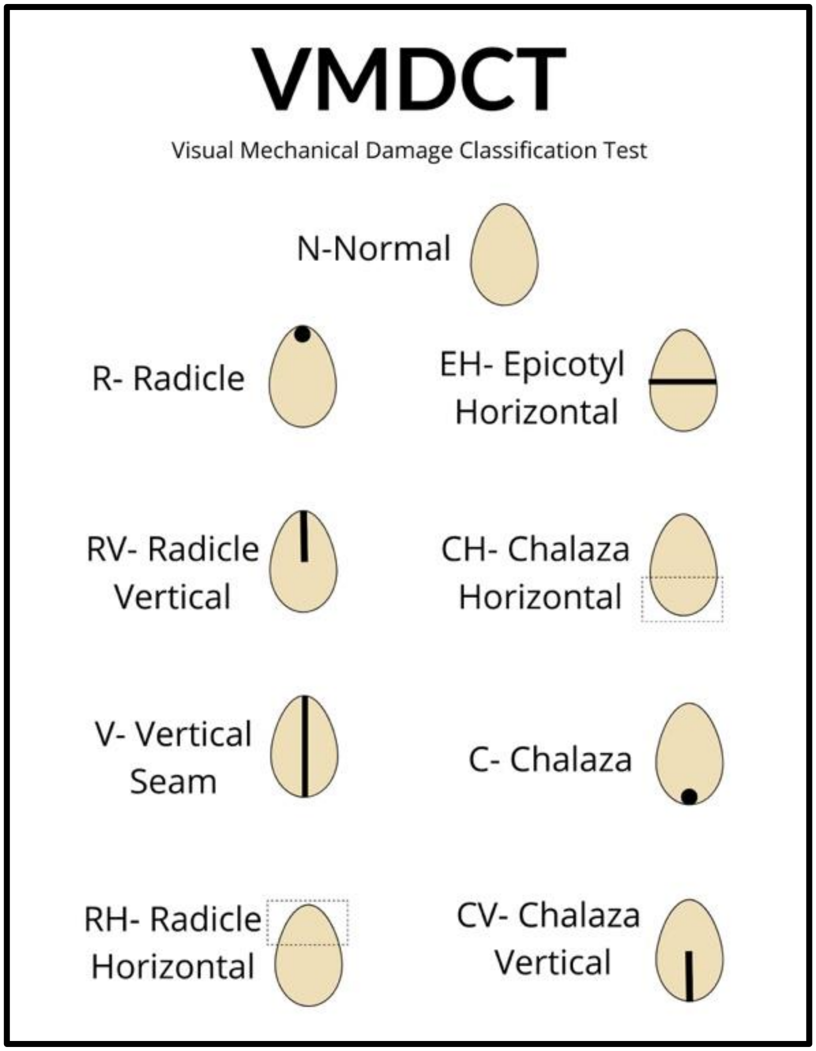

Figure 2. Visual mechanical damage classification test (VMDCT) showed the nine categories of seed damage when mechanically harvested and processed.

The categories represented regions of the seed and internal embryo that may be impacted when those areas are affected. These categories do not represent the severity of damage unless paired with the mechanical damage categories very damaged, damaged, or pinhole. Figure 3 shows visual representations of the categories of damage.

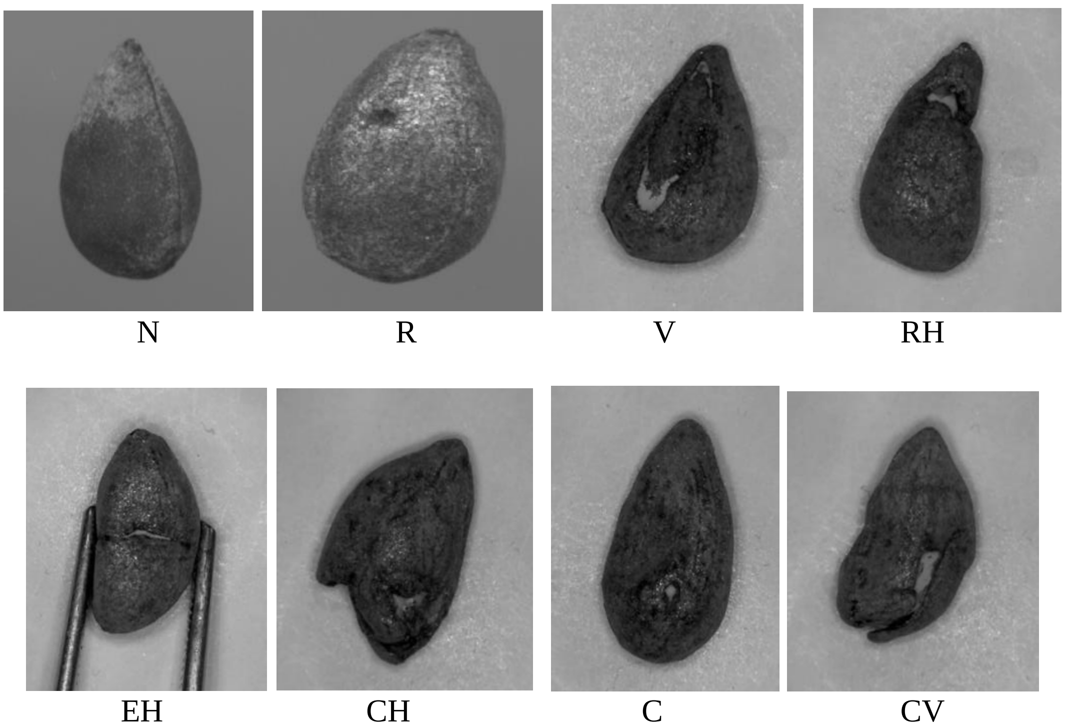

Figure 3. Visual Mechanical Damage Classification Test (VMDCT) visual examples in the categories: Normal (N), Radicle (R), Vertical Seam (V), Radicle Horizontal (RH), Epicotyl Horizontal (EH), Chalaza Horizontal (CH), Chalaza (C), and Chalaza Vertical (CV).

Overall, the collected data creates a database of information on each seed to be used to determine the initial visual health of the seed and how that plays a significant role in the overall outcome of the seedling and cotton plant that you would expect to see in the field. This analysis used JMP software, Version 17, SAS Institute Inc., setting significance at a p-value < 0.05.

Where the ANOVA showed significant differences, pairwise comparisons were made using t-tests to explore specific group differences.

The results are presented as means, and significant differences between treatment groups are denoted using different letters, with groups not sharing the same letter differing significantly ($p < 0.05$).

Results and Discussion

In developing techniques to examine the relationship between seed physiology and mechanical damage more closely, positional location can predict a more accurate representation of the seedling's vigor. This information can be gathered to determine whether certain levels of damage are of no consequence when planted versus those seeds that should be more carefully removed or adjusted for at planting. The classification methods were determined in this study to understand the seed's typical damage patterns.

The study represented each category of damage and locational damage based on what was included in the random sample. The very damaged category was found to have significantly less germination than the other damage categories on days 4, 7, and 12 for both warm and cool germination tests. No other categories were shown to have significant differences in germination rates. From the sample in Figure 4, all categories were at varying rates across the damage location.

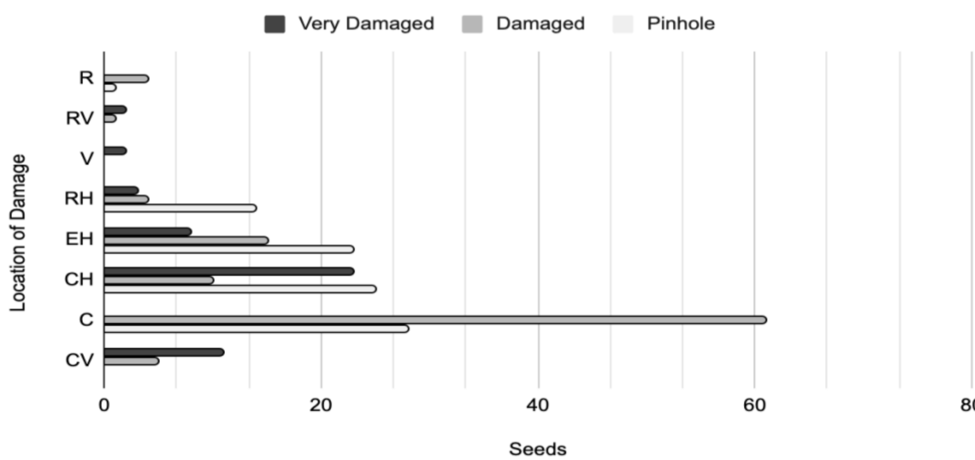

Figure 4. **The mechanically damaged seeds across the nine categories of damage locations** found on the cotton seed. The population of the seeds reported in this figure is for a total of 468 seeds. Normal (N), Radicle (R), Radicle Vertical (RV), Vertical Seam (V), Radicle Horizontal (RH), Epicotyl Horizontal (EH), Chalaza Horizontal (CH), Chalaza (C), Chalaza Vertical (CV).

The locational categories for cool germination that were most prevalent among the seeds that reached the germination level (measuring greater than 4 cm based on AOSA standards) were normal, chalaza, and chalaza horizontal. There was little to no germination in the seeds damaged on the radicle end of the seed across the study, as shown in Table 1. If the seeds damaged on the radicle end continuously fail to germinate, this could indicate that the damage to this area is more detrimental than other areas of the seed, which can help determine the actual severity of damaged seeds in the bag.

As seen in Table 1 for cool germination, day 4 could have been a better indicator of seed vigor as no seeds germinated during this amount of time. Days 7 to 12 increased germination rates across all categories by an average of 54%.

Table 1. Cool Germination tests were read on days 4, 7, and 12, representing the seed germinated by the AOSA standard of greater than 4 cm. No seeds germinated on day 4. Showing the total germination rate for each damage category and the count of seeds, where the total population was 234 seeds.

Cool Germination Results of Seedings Over 4 cm* Damage Locations										
Categories	N	R	RV	V	RH	EH	CH	C	CV	Germination %
DAY 7 COUNT										
VD	0	0	0	0	0	1	0	2	1	8%
D	0	0	0	0	1	3	0	10	0	34%
P	0	0	0	0	1	5	4	9	0	43%
N	37	0	0	0	0	0	0	0	1	33%
DAY 12 COUNT										
Categories	N	R	RV	V	RH	EH	CH	C	CV	Germination %
VD	0	0	1	0	2	2	8	0	4	68%
D	0	0	0	1	2	7	5	26	1	90%
P	0	0	0	0	5	10	11	14	0	90%
N	101	0	0	0	0	0	0	0	0	85%
SUM (234)	101	0	1	1	9	19	24	40	5	85%
% Germination by each damage location for DAY 12										
	50	0	.5	.5	3.8	4.5	12	20	2.5	

Table 1. Continued

*Note: % Germination percentages were for normal seed. The categories were the following: normal (N), radicle (R), radicle vertical (RV), vertical seam (V), radicle horizontal (RH), epicotyl horizontal (EH), chalaza horizontal (CH), chalaza (C), chalaza vertical (CV).

Table 2. Cool Germination test mean separations for germination averages for each VMDCT category read on days 4, 7, and 12, representing the seed germinated by the AOSA standards for cool germination. The total population was 234 seeds. Means sharing the same letter indicate no statistical significance at a p-value of 0.05.

Average Warm Germination Seed Damage Location					
VMDCT Type	Day 7	Mean	VMDCT Type	Day 12	Mean
N	17.52	a	N	43.16	a
C	4.49	b	C	8.54	b
CH	0.57	c	CH	2.70	c
EH	1.28	c	EH	3.42	c
CV	0.22	c	CV	1.07	c
RH	0.29	c	RH	1.28	c
V	0	c	V	0.22	c
R	-	-	R	-	-
RV	0	c	RV	0.43	c

Normal (N) type seeds show the most rapid growth patterns, with radical vertical (RV) showing the least amount of germination (Table 2). Over the 12 days of germination, normal seeds had consistent significance compared to the other categories, along with chalaza (C) on both days 4, 7, and 12. Based on this information, categories N and C are the least severe and least impactful on germination rates since they are not connected to the other locational categories. The average germination increased over the 12 days; however, those that did not increase can indicate that seeds were impacted with damage and where the damage locations were on the seed.

As seen in Table 2 for warm germination, day 4 was a good indicator of seed vigor; however, day 7 showed a higher germination rate across more categories. The locational categories were not found to be statistically different on day 7.

The locational categories for warm germination that were most prevalent among the seeds that did reach the level of germination were N, C, CH, and EH. The highest germination rates were seen in the damage locations of normal and both chalaza and chalaza horizontal. The lowest germination rates were seen in the locational categories R and RV.

On day 4 of the study's warm germination portion, no significant difference was found between the locational categories. On Day 7, there was no significance between the different locational categories; on Day 12, there was also no significance between the locational categories, all checked with a p-value of 0.05.

Table 3. Warm Germination Rates on days 4, 7, and 12 representing the seed germinated by the AOSA standard of a hypocotyl length greater than 4 cm. Showing the total rate of germination for each damage category and what seeds with a known physiological injury were able to germinate. Germinated seeds percentage were calculated with an n= 246.

Warm Germination Results of Seedings Over 4 cm*										
	Damage Locations									
Categories	N	R	RV	V	RH	EH	CH	C	CV	Germination %
DAY 4 COUNT										
VD	0	0	0	0	0	2	10	0	4	64%
D	0	0	1	3	2	7	4	23	2	79%
P	0	1	0	0	7	9	13	12	0	89%
N	118	0	0	0	0	0	0	0	0	89%
DAY 7 COUNT										
Categories	N	R	RV	V	RH	EH	CH	C	CV	Germination %
VD	0	0	1	0	1	3	10	0	5	80%
D	0	0	1	3	2	8	5	27	3	92%

Table 3. Continued

P	0	1	0	0	7	11	13	14	0	95%
N	116	0	0	0	0	0	0	0	0	37%
DAY 12 COUNT										
Categories	N	R	RV	V	RH	EH	CH	C	CV	Germination %
VD	0	0	1	0	1	4	10	0	5	84%
D	0	0	1	3	2	8	5	29	3	96%
P	0	1	0	0	8	11	13	14	0	97%
N	118	0	0	0	0	0	0	0	0	99%
SUM (246)	118	1	2	3	11	23	28	43	8	96%
% Germination by each damage location for DAY 12										
	50	.4	.8	1.2	4.6	9.7	11.8	18	3.3	
SUM (246)	118	1	2	3	11	23	28	43	8	96%
% Germination by each damage location for DAY 12										
	50	.4	.8	1.2	4.6	9.7	11.8	18	3.3	

*Note: % Germination percentages were for seedlings with hypocotyl length > 4 cm. The categories were the following: normal (N), radicle (R), radicle vertical (RV), vertical seam (V), radicle horizontal (RH), epicotyl horizontal (EH), chalaza horizontal (CH), chalaza (C), chalaza vertical (CV).

Table 4 Warm Germination test mean separations for germination averages for each VMDCT category read on days 4, 7, and 12, representing the seed germinated by the AOSA standards for warm germination. The total population was 234 seeds. Means sharing the same letter indicate no statistical significance at a p-value of 0.05.

Average Warm Germination Seed Damage Location								
VMDCT Type	Day 4	Mean	VMDCT Type	Day 7	Mean	VMDCT Type	Day 12	Mean
N	43.85	a	N	47.13	a	N	47.95	a
C	7.18	b	C	8.41	b	C	8.82	b
CH	3.69	c	CH	3.83	c	CH	3.69	c
EH	2.46	cd	EH	3.01	cd	EH	3.01	c
CV	1.23	cd	CV	1.64	cd	CV	1.64	c
RH	1.23	cd	RH	1.37	cd	RH	1.64	c
V	0.62	cd	V	0.62	cd	V	0.62	c

Table 4 Continued

R	0.41	cd	R	0.41	cd	R	0.41	c
RV	0.21	d	RV	0.21	d	RV	0.41	c

Normal (N) type seeds show the most rapid growth patterns, with radical vertical (RV) showing the least amount of germination (Table 4). Over the 12 days of germination, normal seeds had consistent significance compared to the other categories, along with chalaza (C) on both days 4, 7, and 12. Based on this information, categories N and C are the least severe and least impactful on germination rates since they are not connected to the other locational categories. The average germination increases over the 12-day period, indicating that seeds impacted by damage germinate at different rates depending on the location of the damage.

Hypocotyl length was another factor used to observe vigor across the locational damage categories. All seeds were measured on days 4, 7, and 12. Table 5 shows the differences between average lengths. For warm germination, looking at 7 days gives the best estimate of vigor since the number of longer seedlings steadily increased. From 4 to 7, an average of 6 cm, and from 7 to 12, an average of 1.2 cm. For cool germination, it increased the most on day 12. Length in the cool germinated seedling increased the most on day 12, compared to 7 and 4. With an average increase of 2.7 cm from 4 to 7 days and 5 cm for each category from 7 to 12 days.

Length to determine a healthy seedling was a way to see if locational damage impacted seedling growth and development. The WinSEEDLE imaging technology proved helpful as the full life cycle of the germinated seed was traced back to the beginning. Patterns of damage were seen on the cotyledons produced from the damaged seeds in the same pattern shown on the seed at planting. Since the immature cotyledons are present within the seed, the impact can be directly seen in the damage patterns. In the case of damaged cotyledons, the impact on growth and development can be severe in a field setting. With compromised cotyledons, the potential rate of photosynthesis is decreased with less leaf surface area present as the stored energy in the embryo is exhausted.

Table 5. Warm and cool germination lengths on day 7 represented the seed germinated by the AOSA standards. The average length of each damage category for warm and cool germination lengths on day 7 for the seed germinated according to the AOSA standards.

Warm Day 7 Length			Cool Day 7 Length	
Category	Length (cm)		Category	Length (cm)
N	15.97 A		N	9.24 AB
EH	14.96 AB		EH	8.35 B
CV	14.74 AB		CV	6.10 B
R	14.50 ABC		R	na
CH	14.36 AB		CH	7.95 B
V	14.10 ABC		V	4.75 AB
C	14.99 B		C	10.77 A
RH	13.45 ABC		RH	8.97 AB
RV	5.95 CD		RV	na-

*Note: Letters connected by the same letter indicate no significant difference in average lengths when checked with a p-value of 0.05.
The categories were the following: normal (N), radicle (R), radicle vertical (RV), vertical seam (V), radicle horizontal (RH), epicotyl horizontal (EH), chalaza horizontal (CH), chalaza (C), chalaza vertical (CV).

Root development over the 12 days was also recorded in the images taken by the WinSEEDLE scanner; advanced image analysis technology allowed for the images to be tracked. Figure 5 shows an example of the developmental pathway the images followed.

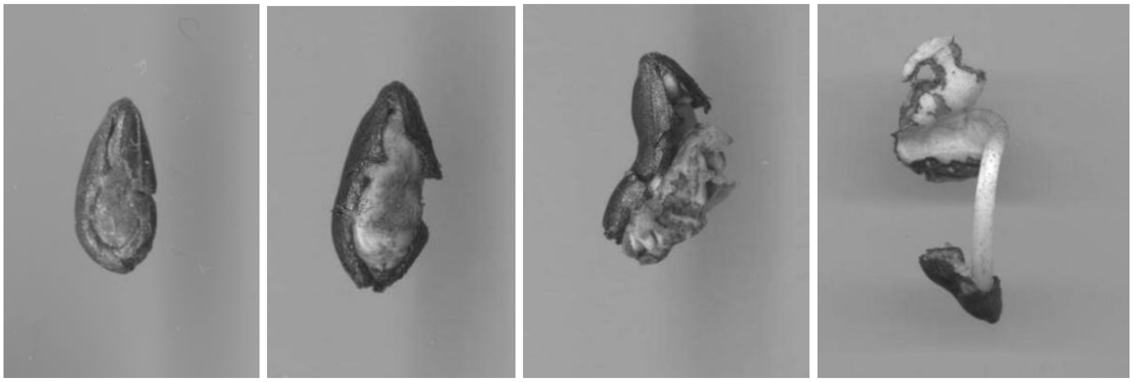

Figure 5. A vertical (V) seam damaged the seed as it progressed from days 0, 4, 7, and 12, displayed from left to right. There was a lack of root growth, and the cotyledons suffered damage within the embryo and necrosis as it attempted to develop.

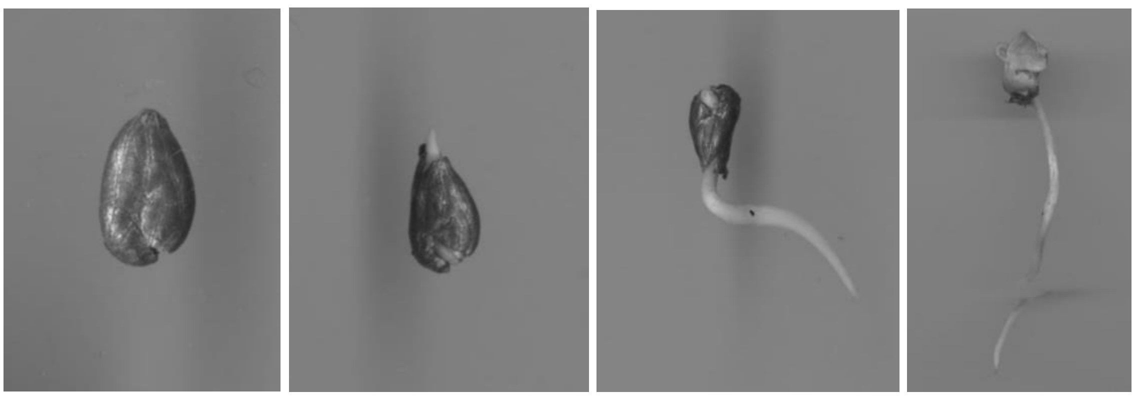

Figure 6. A chalaza vertical (CV) damaged seed and seedling as it progressed from days 0, 4, 7, and 12 displayed from left to right. Damage to the cotyledons within the embryo.

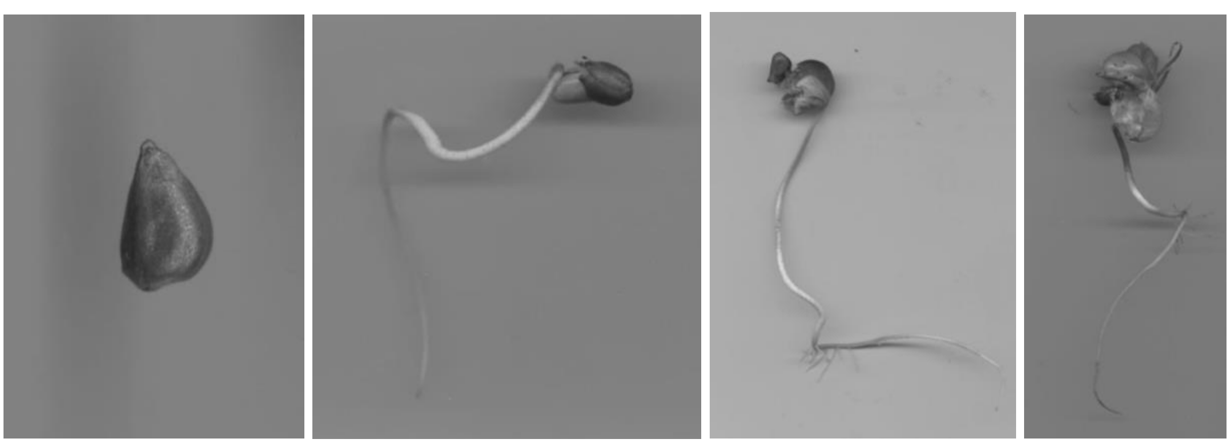

Figure 7. A chalaza horizontal (CH) damaged seed and seedling as it progressed from days 0, 4, 7, and 12 displayed from left to right. Damage to the cotyledons within the embryo.

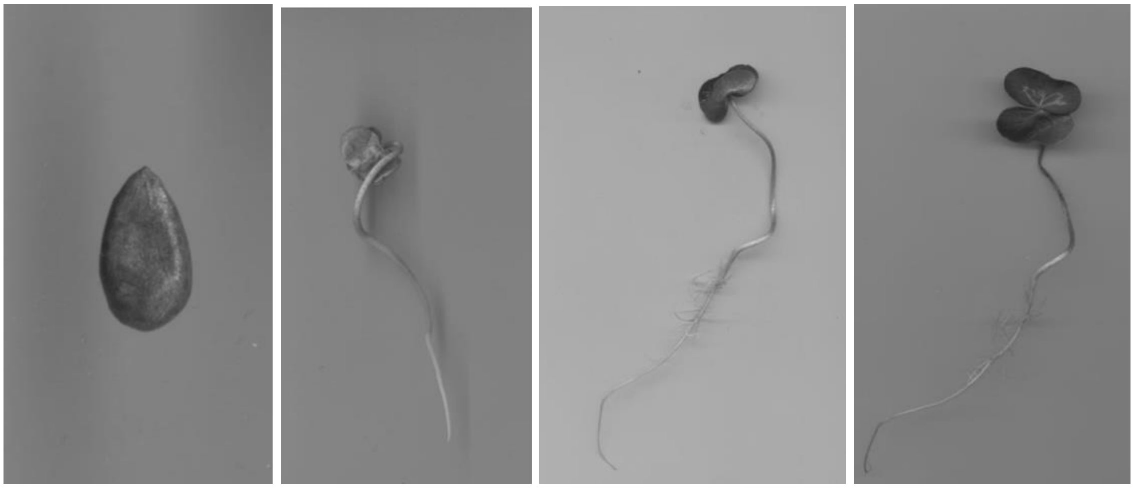

Figure 8. An epicotyl horizontal (EH) damaged seed and seedling as it progressed from days 0, 4, 7, and 12 displayed from left to right. The necrosis on day 12 was likely due to exposure to warm and moist conditions.

Seedling vigor, as defined by radicle length, was another indicator monitored to determine seed health and damage location's impact on performance. At each stage of observation, seedlings were sorted into one of seven categories so that changes in development could be tracked as a measurement tool and predictor of field performance. Those seven categories were dead, hard, gray/decayed, short (< 4 cm), weak (4 to 8 cm), and strong (>8 cm). The categories used were developed from the standards established by the company that provided the sample. Seeing the progression of seedlings throughout the 12 days provides evidence of resilience.

For warm germination, vigor was reported in Table 6, suggesting that looking at 7 and even 12 days gives the best estimate of vigor since the number of longer seedlings increased. Vigor in the cool germinated seedling was reported in Table 5; however, it increased the most on day 12, compared to 7 and 4.

Table 6. Warm germination vigor categories of each damage location across days 4, 7, and 12. Categories across damage locations were not statistically significant at a p-value of 0.05.

Warm Germination Vigor at 12 days
Day 4- Damage Locations

Table 6. Continued

Categories	N	R	RV	V	RH	EH	CH	C	CV
Dead	0	0	0	0	1	1	1	0	0
Hard	0	0	0	0	0	0	0	0	0
Gray/Decayed	0	0	0	0	0	0	0	1	0
Short (<4 cm)	11	1	2	2	3	6	6	6	3
Weak (between 4 & 8 cm)	32	1	1	3	7	8	8	11	4
Strong (> 8 cm)	75	0	0	0	1	9	15	29	3
Day 7- Damage Locations									
Categories	N	R	RV	V	RH	EH	CH	C	CV
Dead	0	0	0	0	1	1	2	1	1
Hard	0	0	0	0	0	0	0	0	0
Gray/Decayed	0	0	0	0	0	0	0	0	0
Short (<4 cm)	0	0	1	0	1	2	3	1	2
Weak (between 4 & 8 cm)	0	0	0	0	0	1	2	0	0
Strong (> 8 cm)	116	2	2	5	11	22	24	44	8
Day 12- Damage Locations									
Categories	N	R	RV	V	RH	EH	CH	C	CV
Dead	8	0	0	0	1	1	4	8	2
Hard	0	0	0	0	2	0	32	0	0
Gray/Decayed	18	0	0	0	1	1	7	19	3
Short (<4 cm)	1	0	0	0	0	0	1	1	0
Weak (between 4 & 8 cm)	0	0	1	0	0	1	3	2	0
Strong (> 8 cm)	117	2	2	5	11	23	24	43	9

Note: The categories were the following: normal (N), radicle (R), radicle vertical (RV), vertical seam (V), radicle horizontal (RH), epicotyl horizontal (EH), chalaza horizontal (CH), chalaza (C), chalaza vertical (CV).

Table 7. Cool germination vigor categories of each damage location across days 4, 7, and 12. Categories across damage locations were not significant at a p-value of 0.05.

Cool Germination Vigor at 12 days									
Day 4									
	Damage Locations								
Categories	N	R	RV	V	RH	EH	CH	C	CV
Dead	0	0	0	0	0	1	0	0	0
Hard	0	0	0	0	0	0	0	0	0
Gray/Decayed	0	0	0	1	0	4	4	0	2
Short (<4 cm)	119	0	1	1	10	18	24	43	5
Weak (between 4 & 8 cm)	0	0	0	0	0	0	0	0	0
Strong (>8 cm)	0	0	0	0	0	0	0	0	0
Day 7									
Categories	N	R	RV	V	RH	EH	CH	C	CV
Dead	1	0	0	0	0	2	0	0	0
Hard	0	0	0	0	0	0	0	0	0
Gray/Decayed	1	0	0	0	0	0	0	0	0
Short (<4 cm)	78	0	1	2	8	14	23	22	6
Weak (between 4 & 8 cm)	32	0	0	0	2	7	5	15	1
Strong (>8 cm)	8	0	0	0	0	2	0	6	0
Day 12									
Categories	N	R	RV	V	RH	EH	CH	C	CV
Dead	3	0	0	0	0	3	2	3	0
Hard	1	0	0	0	0	0	0	0	0
Gray/Decayed	0	0	0	0	0	1	5	0	1
Short (<4 cm)	14	0	0	1	1	4	3	0	2
Weak (between 4 & 8 cm)	32	0	1	1	4	7	11	10	3
Strong (>8 cm)	69	0	0	0	5	12	13	30	2

Note: The categories were the following: normal (N), radicle (R), radicle vertical (RV), vertical seam (V), radicle horizontal (RH), epicotyl horizontal (EH), chalaza horizontal (CH), chalaza (C), chalaza vertical (CV).

Vigor categories such as these are less often used in measuring seedling health. The industry focuses more on warm germination rates. However, the best indicator of seedling performance under optimal conditions is found on germination day 7 when looking at all damage categories and the locational position of damage.

Conclusions

A standard classification test was developed to determine the impact of visual mechanical damage based on the location of the damage on the seed coat. Categories displayed distinct growth abnormalities found within each location. A high-power scanner was used to aid in the identification and classification of visual mechanical damage categories. Standard germination practices sufficed to measure the VMD categories; however, it would be imperative to repeat this study using multiple varieties in the field and greenhouse growing conditions. The small sample size contributed to the significance rates, as the more severe categories were less prevalent in the sample and could not be equally distributed between warm and cool germination studies because of lower prevalence. The sample size was provided by a third party and was limited to the amount given.

Knowing the types of damage that are more detrimental to seed performance can help determine where the more severe damage occurs in the mechanical processing of seeds. Based on the mean separations, the locations of N and C were highest performing and statistically different from the other categories. Using the VMDCT to mitigate VMD impact in production can be a way to decrease the number of seed batches thrown out. If a lot of seeds have a higher rate of VMD, in which there are the majority of C-impacted seeds, it would be more beneficial than a high population of seeds injured on the radical end of the seed. Techniques such as sampling seeds at every stage of the process could help mitigate and decrease the rate of damage occurring if it can be measured along the processing route of the seeds. With today's technology and the advancements of machine learning and artificial intelligence, a classification system for cotton seed damage based on location would greatly benefit the seed industry with the potential to remove the most severe damage that could be sold to the farmer.

The classification system reported herein will also help improve other non-destructive methods correlating to cotton seeding vigor. Each seed image can directly track individual seedling performance, and its high-quality image shows the damaged locations. With other non-destructive technologies such as X-ray imaging, a view of the internal seed structures can identify hidden damage (Rode & Sawarker, 2017); the crushing test or mechanical test, controlled pressure to assess seed coat strength and identify compression damage (Fu et al., 2024); optical spectroscopy, a powerful tool to inspect seed, especially in characterizing internal quality (Sunoi et al., 2016); and thermal imaging, a technique for converting the invisible infrared radiation pattern of an object into visible images for feature extraction and analysis (Rahman & Cho, 2016) would benefit from this study. Most important is knowing the overall percentages of the occurrence of specific damage locations found in commercially sold bags of seed. Another limitation of this study is that it only provides data for one commercial variety; however, it did provide the first classification system created for damaged cotton seeds, and the cotton seed industry would likely benefit from a standardized methodology and an understanding of how VMD damage locations correlate to seed quality and its vigor on a larger scale.

Cotton seeds possess remarkable strength, requiring significant force to crack or fully break into the embryo. Within the embryo, several crucial components are situated primarily at the polar ends of the seed, notably the chalaza and the radical ends (Figure 1). While it remains uncertain which section of the seed responds more adversely to damage, preliminary observations indicated that damage inflicted on the radical results in a reduced survival rate compared to damage occurring elsewhere on the seed.

In developing techniques to look more closely at the relationship between seed physiology and mechanical damage, a more accurate representation of seedling vigor can be predicted by the damage's variety and positional location. The information can be gathered to determine if certain levels of damage are of no consequence when planted versus those seeds that should be more carefully removed or adjusted for at planting. The classification methods reported in this study provided information to observe what location has the most damage to the embryo and how the presence of damage impacts the seedling's health. Hopefully, in the future, this information can be expanded upon with more varieties and larger samples.

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APPENDICES

Appendix A. The analysis of variance of visual mechanical damage (VMD) warm germination percentage and oil percentage.

Regression of VMD Warm Germination Percentage to Oil Percentage				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3127.910	3127.91	6.3896
Error	35	17133.591	489.53	Prob > F
C. Total	36	20261.501		0.0161
Parameter Estimates				
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-4.540411	24.23748	-0.19	0.8525
Oil Percentage	3.1832898	1.259329	2.53	0.0161

Appendix B. The stepwise regression model for the stand counts at 7 DAP percentages using visual mechanical damage (VMD) warm germination percentage and seeds per lb as variables.

Effect Summary			**Summary of Fit**	
Source	**Logworth**	**P-Value**	**RSquare**	**0.6289**
VMD Warm Germination %	2.900	0.00126	**RSquare Adj**	**0.5852**
Seeds per lb	2.397	0.00401	Root Mean Square Error	9.302496
			Mean of Response	49.25
			Observations (or Sum Wgts)	20
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	2	2492.6308	1246.32	14.4022
Error	17	1471.1192	86.54	Prob > F
C. Total	19	3963.7500		0.0002*
Parameter Estimates				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	43.006666	21.48204	2.00	0.0615
Seeds per lb	-0.004204	0.001264	-3.33	0.0040*
VMD Warm Germination %	0.8412457	0.217979	3.86	0.0013*

Appendix C. Stepwise regression model for the stand counts at 10 DAP percentage using visual, mechanical damage, warm germination, and oil percentage as variables.

Effect Summary			Summary of Fit	
Source	**Logworth**	**P-Value**	**RSquare**	**0.5493**
VMD Warm Germination %	3.757	0.00017	**RSquare Adj**	**0.522819**
Oil %	2.100	0.00795	Root Mean Square Error	13.68977
			Mean of Response	53.08108
			Observations (or Sum Wgts)	37
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	2	7766.823	3883.41	20.7215
Error	34	6371.933	187.41	Prob > F
C. Total	36	14138.757		<0.0001*
Parameter Estimates				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	-17.08475	15.0014	-1.14	0.2628
VMD Warm Germination %	0.4406552	0.104586	4.21	0.0002*
Oil %	2.38944	0.847338	2.82	0.0079*

Appendix D. Stepwise regression models for the stand counts at 7 DAP and 10 DAP percentages using visual mechanical damage (VMD) warm germination percentage, the VMD percentage for very damaged (VD) plus damaged (D) seeds, and seeds per lb as variables.

Effect Summary					**Summary of Fit**				
Source	**Logworth 10 DAP%**	**P-Value 10 DAP%**	**Logworth 7 DAP%**	**P-Value 7 DAP%**		**10 DAP %**	**7 DAP %**		
VMD Warm Germination %	3.126	0.00075	3.272	0.00053	**RSquare**	**0.418643**	**0.678169**		
Seeds per lb	0.236	0.58048	2.744	0.00180	**RSquare Adj**	**0.358502**	**0.617825**		
VMD, VD+D%	0.192	0.64202	0.863	0.13698	Root Mean Square Error	15.75468	8.929092		
					Mean of Response	50.7878	49.25		
					Observations (or Sum Wgts)	33	20		
Analysis of Variance					**Parameter Estimates**				
Stand Counts 10 DAP %									
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Term**	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Model	3	5153.429	1727.81	6.9611	Intercept	41.444526	28.06467	1.48	0.1505
Error	29	7198/087	248.21	**Prob > F**	VMD, VD + D%	-0.323136	0.687826	-0.47	0.6420
C. Total	32	12381.515		0.0011*	Seeds per lb	-0.002531	0.004632	-0.56	0.5805
					VMD Warm Germination %	0.4945033	0.13185	3.77	0.0007*
Stand Counts 7 DAP									
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Term**	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Model	3	2688.0911	896.030	11.2385	Intercept	30.206649	22.1814	1.36	0.1921
Error	15	1275.6589	79.729	**Prob > F**	VMD, VD + D%	0.8632751	0.551369	1.57	0.1370
C. Total	18	3963.7500		0.0003*	Seeds per lb	-0.010283	0.002753	-3.73	0.0018*
					VMD Warm Germination %	0.9668881	0.22409	4.31	0.0005*

Appendix E. Stepwise regression model for the stand counts at 10 DAP percentage using NC State's warm germination percentage, the visual mechanical damage (VMD) percentage for very damaged (VD) plus damaged (D) plus pinhole (P) seeds, and the VMD percentage for VD seeds as variables.

<table>
<tr><th colspan="3">Effect Summary</th><th colspan="2">Summary of Fit</th></tr>
<tr><th>Source</th><th>Logworth</th><th>P-Value</th><th>RSquare</th><th>0.541196</th></tr>
<tr><td>NCSU
Warm Germination %</td><td>4.9646</td><td>0.00001</td><td>RSquare Adj</td><td>0.499487</td></tr>
<tr><td>VMD, VD%</td><td>1.304</td><td>0.04970</td><td>Root Mean Square Error</td><td>14.02046</td></tr>
<tr><td>VMD, VD + D + P%</td><td>0.961</td><td>0.10930</td><td>Mean of Response</td><td>53.08108</td></tr>
<tr><td></td><td></td><td></td><td>Observations (or Sum Wgts)</td><td>37</td></tr>
<tr><th colspan="5">Analysis of Variance</th></tr>
<tr><th>Source</th><th>DF</th><th>Sum of Squares</th><th>Mean Square</th><th>F Ratio</th></tr>
<tr><td>Model</td><td>3</td><td>7651.842</td><td>2550.61</td><td>12.9754</td></tr>
<tr><td>Error</td><td>33</td><td>6486.915</td><td>196.57</td><td>Prob > F</td></tr>
<tr><td>C. Total</td><td>36</td><td>14138.757</td><td></td><td><.0001*</td></tr>
<tr><th colspan="5">Parameter Estimates</th></tr>
<tr><th>Term</th><th>Estimate</th><th>Std Error</th><th>t Ratio</th><th>Prob>|t|</th></tr>
<tr><td>Intercept</td><td>19.585649</td><td>9.505676</td><td>2.06</td><td>0.0473*</td></tr>
<tr><td>VMD, VD + D + P%</td><td>1.9190723</td><td>1.166061</td><td>1.65</td><td>0.1093</td></tr>
<tr><td>VMD, VD%</td><td>-2.784536</td><td>1.366742</td><td>-2.04</td><td>0.0497*</td></tr>
<tr><td>NCSU
Warm Germination %</td><td>0.4685204</td><td>0.090704</td><td>5.17</td><td><.0001*</td></tr>
</table>

Appendix F. Stepwise regression model for the stand counts at 10 DAP percentage using visual, mechanical damage (VMD) warm germination percentage, the VMD percentage for very damaged (VD) plus damaged (D) plus pinhole (P) seeds, non-damaged seeds percentage, and percentage of oil as variables.

Effect Summary			**Summary of Fit**	
Source	**Logworth**	**PValue**	**RSquare**	**0.591682**
VMD Warm Germination %	3.423	0.00038	**RSquare Adj**	**0.540643**
Oil %	2.433	0.00369	**Root Mean Square Error**	13.43166
Non-damaged %	1.101	0.07916	Mean of Response	53.08108
VMD, VD + D + P %	1.077	0.08367	Observations (or Sum Wgts)	37
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	4	8365.654	2091.41	11.5926
Error	32	5773.102	180.41	**Prob > F**
C. Total	36	14138.757		<.0001*
Parameter Estimates				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	957.77108	538.7386	1.78	0.0849
Non-damaged %	-9.80810509	5.408724	-1.81	0.0792
VMD Warm Germination %	0.4128383	0.103922	3.97	0.0004*
Oil %	2.673828	0.853598	3.13	0.0037*
VMD, VD + D + P %	-9.704724	5.435447	-1.79	0.0837