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Factors Affecting Within-plant Variation of Cotton Fiber Quality and Yield

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FACTORS AFFECTING WITHIN-PLANT VARIATION OF COTTON FIBER QUALITY AND YIELD

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
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in

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by
Matthew Oliver Indest
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Table of Contents

Abstract

Cotton is sold by weight, but a bale's lint price per pound is determined by its fiber quality profile. Cotton quality is defined by a set of standardized properties (length, strength, elongation, uniformity, color, trash, and micronaire) collected on every United States bale. Each cotton fiber is the remnant of a single cell which upon harvest exists as a dry, hollow tube of crystalline cellulose. The length, perimeter, and thickness are a fiber's physical dimensions. These dimensions influence both the mechanics involved in yarn spinning and the quality of the yarn produced. Genetic and environmental factors affect the development and consequently, the final properties of cotton crops. However, information is lacking about the degree of influence they impart, especially on fiber perimeter (fineness) and cell wall thickness (maturity), both components of micronaire.

The goals of this dissertation were to: 1) Summarize and review the techniques available to industry to measure fiber perimeter and maturity in order to discuss their advantages and limitations, 2) Validate the use Cottonscope to measure fiber quality variation, 3) Determine the significance of within-plant yield variation, and 4) Determine the significance of within-plant quality variation.

Small differences in micronaire are often indistinguishable, making breeding efforts difficult. With new instruments, selecting for the components of micronaire may increase selection efficiency and genetic gain compared to breeding for micronaire directly. In addition, these results show that yield and quality within genotypes are highly variable, and a significant amount of the variation is attributable to a boll's fruiting site. Substantial bias can be introduced if boll sampling does not consider fruiting position. The results show that plot sampling techniques can greatly influence fiber quality testing results and as a result the effectiveness of genetic selection. The Cottonscope is a very accurate and precise tool for measuring fiber fineness and maturity ratio and improving the interpretation of micronaire. Micronaire had strong correlation with fiber fineness data. Breeding for lower micronaire would be a useful strategy to improve fiber fineness in environments where low fiber maturity is not a problem

Chapter 1 - Fiber quality quantification: fiber fineness and maturity ratio

1.1 Micronaire

Cotton (primarily *Gossypium hirsutum* L. and to a lesser extent *G. barbadense*, *G. arboretum*, and *G. herbacium*) is the most important natural fiber crop in the world; the total economic revenue generated in the United States is over $100 billion. According to the United States Department of Agriculture's "Cotton: World Markets and Trade" report, the U.S. produced 15,000,000 bales of cotton in the 2013-2014 growing season, with only 3,700,000 of the bales being used domestically. The crop was produced on 3.98 million hectares of farm land (Cotton: World Markets and Trade, 2014). Cotton fiber is sold by weight, and the price per bale is determined by its fiber quality profile. The "A" index, which represents the lowest international quotes, averaged 93.29 cents/pound between January-July in 2014, whereas the price received by farmers averaged 79.18 cents/pound (Monthly Prices, 2014).

Fiber quality measurements which are most important to industry have values associated with them that impact pricing. There is a base value, and there are premiums and discounts applied based on a bales deviation from the base value. Historically, fiber quality was determined by hand grading. Graders would feel a sample to determine its relative quality. Greater precision was needed, thus numerous methods have been developed. While these methods were more precise, they are much slower than hand grading. With the exception of color and leaf grades (which are still 'called' by hand), the remaining parameters are measured mechanically by High Volume Instrumentation (HVI). HVI machines are manufactured by Uster Technologies (Memphis, TN). Of the mechanically measured fiber properties, the one with the largest impact on fiber quality pricing is micronaire.

There are many quality parameters associate with cotton fiber and many more instruments and methods to measure them. Each technique has advantages and disadvantages relating to the cost and ease of acquiring the measurements, thus the relative value to cotton scientists and the industry differ. While industry requires an extremely fast turnover for fiber quality assessment and tolerates less precision in measurements, scientists cannot always depend on industry standard methods to conduct research.

1

Micronaire (MIC) is determined by the air flow permeability of a cotton sample. It serves as a swiftly acquired indicator of both fiber fineness and fiber maturity. As an indicator of fineness, it is used to predict spinning efficiency and final yarn thickness. As an indicator of maturity, it is used to predict how consistent dye uptake will be. Micronaire is the most rapid, widely used, and least expensive estimate of fiber fineness and maturity ratio. The ideal fiber profile has finer fibers (lower mtex) and full maturity fibers (higher maturity ratio). Immature fibers have a collapsed lumen due to thin cell walls and low cell wall circularity. This can cause a low MIC bale to be interpreted as fine when it is actually immature. Conversely, high MIC can be misinterpreted as too coarse (high mtex) when the sample maturity is high wherein the lumen does not collapse and cell wall circularity is maintained (Fig 1.1.1 and 1.1.2).
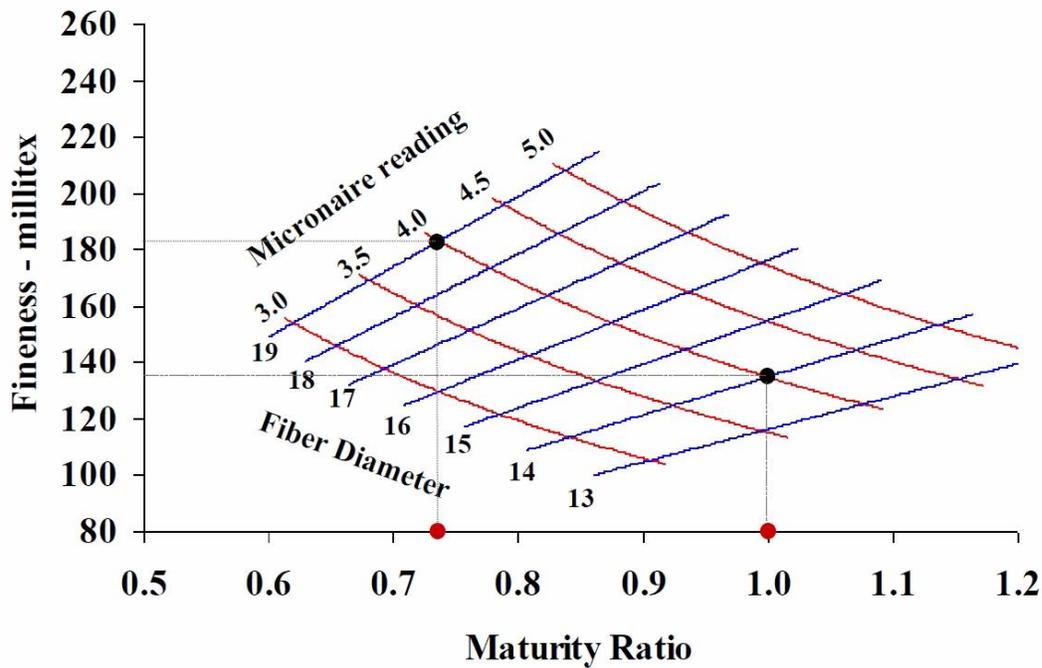
Figure 1.1.1. Relationship between micronaire and fiber fineness (mtex) and maturity ratio according to the Lord equation (Lord, 1956) *Image courtesy of E. Hequet (Texas Tech University, Lubbock, TX)*
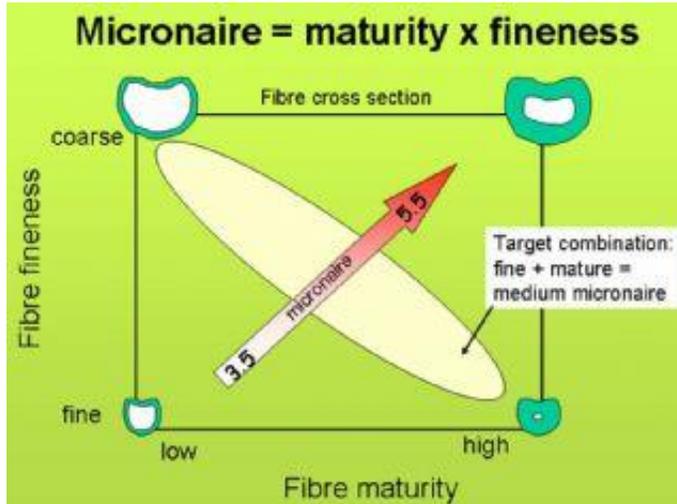
Figure 1.1.2. Ideal range of fiber fineness and maturity
Image courtesy of E. Hequet (Texas Tech University, Lubbock, TX)
https://www.seedquest.com/News/releases/2006/march/15333.htm
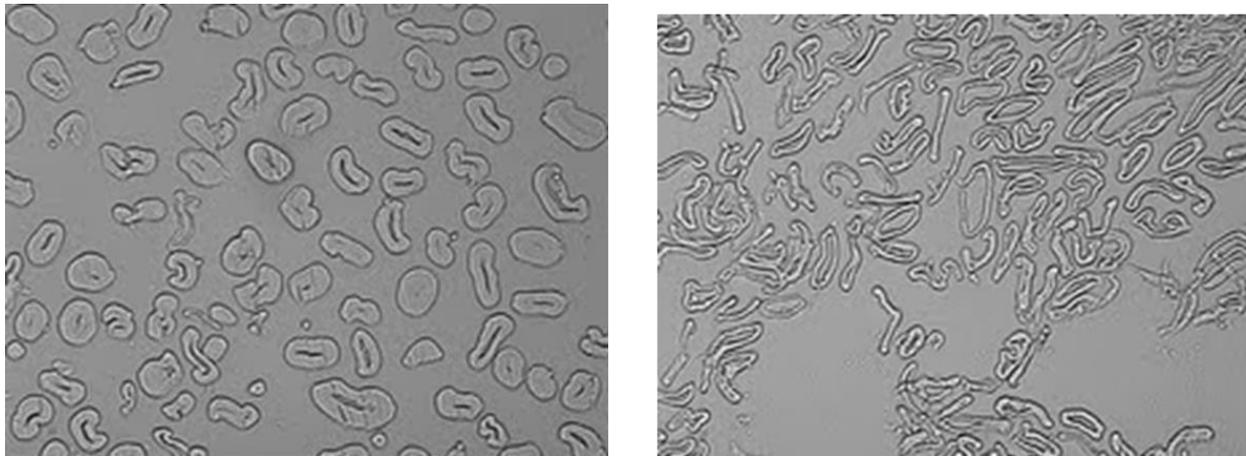
Figure 1.1.3 Cross-sectional Image Analysis micrographs of cotton samples: mature (left) and immature (right) fibers *Images courtesy of J. Moraitis (USDA-ARS, New Orleans, LA)*

Micronaire is an important measurement to the cotton industry, but its interpretation varies both in meaning and value depending on who is asked. Micronaire has been considered ill-suited in estimating fiber fineness because of the significant influence of maturity (Raskopf, 1966; Kloth, 1998; Thibodeaux and Rajasekaran, 1999; Abbott, 2010; Clement et al. 2012). Direct measurements for fiber fineness and maturity ratio greatly improve the ability to predict yarn output and efficiency, but are tedious and costly. The techniques required by industry must combine low cost and rapid quantification. Consequently,

micronaire is the most widely used measurement for indicating these traits despite all evidence of its inadequacies. Improving predictions in spinning equates to more consistent yarn output and better efficiency (Deussen, 1992). Besides the improvement to prediction models that would be achieved, measuring fineness and maturity is of more value to scientists than micronaire. Understanding fiber cell wall development depends on accurate and precise measurements that micronaire is unable to provide due to large sample size and poor accuracy.

The United States loan rates on cotton lint are an illustration of the effect micronaire has on pricing (Table 1.1.1). Discounts are listed in points (1/100th of a cent) per pound. While these discounts do not represent the exact market value of cotton, they do give an indication of the effect micronaire has.

The relationship between fiber quality and the actual price received across growing regions of the United States cotton belt has been studied (Etheridge and Hudson, 1998; Chakraborty and Etheridge, 1999). In these studies, analysis of regression beta values for fiber length, micronaire, and trash were found to be significant; however differences between regions were also noted. This means that these fiber quality parameters were impacting the actual price of cotton. Chakraborty and Etheridge (1999) found that micronaire, as well as length, had a significant impact on the actual price paid for a bale of cotton. Actual discounts attributed to high MIC in the Southern cotton region were estimated at -1053 points/lb.

Table 1.1.1. NCC loan discount and premium schedules 2013 and 2014

Micronaire	Class	Points pound^{-1} adjustment (2013)	Points pound^{-1} adjustment (2014)
<3.4	Low Discount	-950 (<2.4) -900 (2.5-2.6) -605 (2.7-2.9) -340 (3.0-3.2) -170 (3.3-3.4)	-950 -900 -600 -340 -170
3.5-3.6	Low Base	0	0
3.7-4.2	Premium	+15	+15
4.2-4.9	High Base	0	0
>5.0	High Discount	-270 (5.0-5.2) -385 (>5.3)	-285 -410

The impact on price is appreciable, but undesirable micronaire has an effect beyond just the direct effect on price. A bale of cotton with poor micronaire but otherwise ideal fiber quality parameters is likely to remain unselected by buyers entirely. Cotton mills depend on consistency during processing to achieve efficient turnout (Deussen, 1992). Spinning mills can accommodate bales with less than ideal length or strength through blending during laydown; but if a bale's micronaire is too far off, blending it into more ideal bales will not solve the problem (Classification of Upland Cotton, 2015). The effect micronaire has on spinning cannot be overcome by diluting the subpar raw material with suitable fibers. Instead, the occurrence of yarn breakage, or ends-down, will rise resulting in a slower and inefficient run. Time down for a spinning mill is costly; therefore it is cheaper to avoid use of sub-par micronaire bales entirely as opposed to incorporating them during laydown. While micronaire does have an effect on price, the most important impact is on bale utilization (Hake et al., 1990). Discounts may cause a slight decrease in price, but an unsold bale represents a total loss (Chris Delhom, Personal Communication, 8/20/2014).

The USDA is responsible for managing classing offices which evaluate quality for all cotton grown in the United States. The data is released in a periodic report titled *Quality of cotton classed by the classing office* (USDA AMS, 2014). One office, located in Rayville, LA, reported during the week of February 6, 2014 that 43.5% of cotton classed had an average micronaire between 5.0-5.2 and an additional 13.4% above 5.3 (Quality of cotton classed by the classing office, 2014). This data shows that more than half of the cotton passing through Rayville's classing office has a micronaire to which a discount on quality can be assessed. Rayville sampled 341,802 bales during the growing season. The aggregate loss due to micronaire alone amounts to a $2.9 million loss in value (-3.35%) for the cotton which passes through Rayville. As a percent of the total value for individual bales, the amount may seem rather small. However, cotton has continued to increase in micronaire, and the trend is that this will continue causing larger discounts on more cotton bales in the future. This loss not only affects producers and raw cotton buyers but it also carries over to processors and consumers in terms of price, end product

quality, and supply. The loss in cotton value is a loss of efficiency in cotton production. Addressing the issue of rising micronaire early is the best way to prevent further weakening of cotton prices.

1.2 Instrumentation

High Volume Instrumentation (HVI) is a modular system, manufactured by Uster Technologies (Knoxville, TN), which rapidly measures a set of cotton fiber quality traits: length, length uniformity, strength, elongation, micronaire, short fiber content, color, and reflectance. Historically, each trait was quantified by individual instruments until Uster Technologies combined them into a single, high volume system. Based on the Fibronaire instrument, HVI measures MIC by compressing a weighed sample to a specific volume, passing pressurized air through the plug, and measuring the air flow resistance. MIC (whether from HVI or Fibronaire) is unit-less and values range from 2.2 - 8. The property of a cotton sample's air-flow resistance was modelled to represent fiber fineness (Lord, 1956a) It was quickly reported that the sample's maturity ratio was confounding interpretation (Lord, 1956b). Two problems were evident: 1) neither fineness nor maturity ratio is measured directly using air-flow resistance and, 2) micronaire values are not scaled to standard units of pressure. These problems heavily confound interpretation of micronaire. In theory, one can estimate micronaire, fineness, or maturity using the function defined by Lord (1956a), but the errors in prediction are high (Lord, 1956b). Since then, many techniques, adjustments, and instruments have been developed to improve measurements of fineness and maturity ratio or resolve interpretations of MIC.

The Advanced Fiber Information System (AFIS), also a product by Uster Technologies, is a machine that measures fiber quality traits using optical analysis of individualized fibers. Samples are fed into the machine by rollers which open the fiber sample and separate trash. Fibers are individualized, and an air stream passes them across a set of optical sensors which measure each fiber's dimensions. Fiber fineness (linear density) is determined using sensors to optically examine the individualized fiber's shape. Maturity ratio is determined by the degree of light scattering as fibers pass across the light source.

The first image analysis reference methods were established by Boylsten et al. (1993) and required fiber cross sections for accuracy. Longitudinal scans were unsuccessful due to fiber twisting. Cross-sectional image analysis is the reference standard for fiber maturity and fineness instrumentation (Thibodeaux and Rajasekaran, 1999). Micrographs of embedded fiber cross sections can be used to calculate a fiber sample's precise dimensions (Fig. 1.1.3). While this technique is very accurate, it requires expensive equipment, highly skilled technicians, and is a lengthy process.

Until recently, major efforts to address micronaire were not a priority due to technical limitations. Use of micronaire in cotton breeding is to ensure that cultivars are within an acceptable range. Environment is the primary source of variation for micronaire; consequently, gains from selection are slow. In a study of regional variety trials over seven years, Meredith et al. (2012) found micronaire to display the lowest variation due to genetics of all fiber quality traits examined. In the same work, a summary of seven Genotype-Environment Interaction (GEI) studies was compiled which showed MIC having the lowest genetic (18%) and highest environmental variation (66%) (Meredith et al., 2012). Further complicating genetic improvement is that variety selection based on HVI micronaire alone is likely an unsuccessful approach. Its complex relationship to fineness and maturity ratio make it a poor indicator of either trait (Thibodeaux and Rajasekaran, 1999). Historically, methods and instruments to measure fiber maturity and fineness were either slow, costly, and as Montalvo et al. (2007) proved with AFIS, contained significant bias. Modern techniques, which automate the process, provide for more accurate and rapid quantification (Rodgers et al., 2012b)

The Cottonscope® was developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO) of Australia by combining the technology of the SiroMat™ and the Cottonscan™ to measure both fiber fineness and maturity. The Cottonscope is an automated microscope which combines the "cut-and-weigh" method of obtaining fineness (mtex) and a polarized light refraction method of quantifying a fiber sample's maturity ratio (MR). The Cottonscope has proved to be more accurate and precise than HVI as well as less expensive and faster than AFIS (Rodgers et al., 2012a;

7

Paudel et al., 2013). To use the Cottonscope, fiber samples are prepared by chopping with an accompanying knife apparatus to approximately 0.7 mm in length, weighted to 50 ± 0.2mg, and suspended in an ionized water bath compartment. The water bath is agitated to allow individual fibers (snippets) to pass across a microscope lens illuminated with polarized light, their images captured, and dimensions measured. This system provides precise longitudinal scans of cotton fibers which maintain the accuracy of image analysis techniques.

The Cottonscope is a valuable instrument for examining cell wall development. The accuracy and precision capabilities this low cost, small footprint instrument is unmatched. The utility it holds for breeders is still subject to scrutiny, yet it does present great potential. The Cottonscope is unique in that it directly measures fiber maturity and fineness with remarkable agreement to microscopic image analysis at a lower cost and in less time. The instrument presents unique opportunities for investigating cotton fibers. Sample size with the Cottonscope (50mg) is not a limiting factor as with AFIS (500mg) and Fibronaire (3.24g), therefore individual bolls can be measured. The swift measurement of thousands of individualized fibers gives distribution statistics. Examining genotypic differences in sample distributions is more powerful than simply comparing means.

1.3 Importance to Genetic Improvement

Phenotypic variation of a trait is a sum of several components which must be separated before they can be utilized for selection in breeding. Total variance is a sum of genetic variance (G), environmental variance (E), and variance caused by the interaction (GEI) between genetic and environmental factors. It is the genetic variance that is useful in breeding. To estimate the relative proportions of G, E, and GEI it is necessary to conduct properly designed research trials in multiple environments (METs). Environments are usually either locations, years, or some combination of the two. METs are able to provide estimates of these components of variance and can also provide perspective as to a genotype's stability. METs typically require large, concerted efforts to manage and are costly. The resources available to smaller breeding programs may not afford more than a few testing locations for

their varieties. One way to get a better estimate of stability is to analyze distributions in a crop as opposed to comparing means. If yield is compared as a mean value of genotypes, then many years will need to be tested to see how the genotype will respond to the environment. However, it may be possible to examine a genotype's stability in response to environments through the distribution it displays within a single location.

Cotton yield has seen great improvement, but as yield has increased, so has the average micronaire value of commercial varieties. Coarse, mature fibers weigh more per unit volume than fine, immature fibers, but the market utility of high MIC fiber is limited. This trend is supported by the positive linkage of yield with fiber maturity (Clement et al., 2012). Desalegn et al. (2009) found that fiber quality, strength most notably, had a large negative genetic association to lint yield. However, fiber fineness (indicated by MIC) proved the opposite and was positively correlated to yield.

Heritability estimates for MIC demonstrated potential for selection in one study (May, 1998) but estimates were low in another (May and Jividen, 1999). The interaction within a fiber sample's fineness and maturity ratio and their independent heritability make it difficult to know how breeding selection using MIC alone will affect fineness and maturity. Heritability determines the population size needed to effectively making selection gains (Poehlman, 1987). Examining the degree of influence each component trait is related to MIC will provide more insight into how breeding goals should be focused. Without this information, HVI MIC is not reliable enough for breeders to make selection pressure.

Selection strategies could be used to curb the current trend towards increasing micronaire if its components factors were better understood and more easily measured. The Cottonscope is a new instrument for fast, economical, precise, and accurate measurement of the components of micronaire. The Cottonscope has potential for utilization by breeders. The strategy would begin by the partitioning of variance into G, E, GEI components to assess the degree to which each influences a crop's micronaire, fineness, and MR. Secondly, a direction for selection for fiber fineness and maturity is required so that

changes in MIC are goal oriented with regard to these traits since the relationship between these three

traits is not linear. Third, stability must be analyzed so that gains are neither environmentally specific nor

do they decay when exposed to uncontrollable environmental stress. Environmental factors can only be

controlled in part by cultural practices; therefore it is important to maximize the genetic potential of

varieties so that their performance is both optimal and reproducible.

The research presented herein will determine how cotton breeding can be used to address the

problems associated with micronaire. The objectives are to examine cotton fiber quality parameters

specifically micronaire, and its components: fiber fineness and maturity.   As a result, selection for

varieties with improved micronaire can be focused and effective.

1.4 Objectives
1. Validate the use of the Cottonscope to measure fiber quality variation.

2. Determine the significance of within-plant yield variation.

3. Determine the significance of within-plant quality variation.

Chapter 2 - Micronaire, fineness, and maturity ratio measurements for eleven genotypes grown in Louisiana environment

2.1 Introduction

Cotton breeding efforts have focused mainly on increasing fiber yield, overcoming biotic and abiotic stresses, and improving fiber quality. These problems are addressed in numerous ways, but can prove difficult if approached simultaneously since the improvement of one trait is, more frequently than not, correlated with a decline in another. For example, focusing solely on yield may neglect fiber quality traits to the point of limiting post-harvest utility. A negative association has been defined between yield and many fiber quality parameters excluding fiber micronaire, fineness, or maturity. This effect was demonstrated in both U.S. and Australian high quality breeding material (Clement et al., 2012).

The association between multiple traits may be due to pleitrophy, epistasis, or linkage. In the case of pleitrophy where one gene seemingly has an effect on multiple traits, there may be little hope for improvement. Here inter-trait linkages such as common pathway correlations (positive or negative) as well as environmental interactions in various fiber quality measures may limit both the potential for genetic gain and economic return that can be achieved by selecting on individual quality measures. For both epistasis and linkage, the use of large recombinant populations to achieve new gene combinations could lead to new, favorable gene combinations that allow for simultaneous improvement across multiple traits. There are few examples where this has been achieved between fiber yield and fiber quality in cotton. Cotton yields have broken historical records due to improved varieties and management practices; however, varieties obtaining these yields can be sacrificing some important quality parameters which limit their market demand. As upland cotton lint yield has increased, so has the average MIC value. Coarse, mature fibers weigh more per unit volume than fine, immature fibers, but the demand for high MIC fiber is limited. This trend is supported by the positive linkage with fiber maturity (Clement et al., 2012).

The Regional Breeder's Testing Network (RBTN) (www.cottonrbtn.com) is a public multi-location variety trial where breeders can submit varieties for performance evaluation across the diversity

of the United States Cotton Belt. The 2012 test was structured as a randomized complete block design comprised of thirty one entries and three competitive control lines which were grown at fourteen locations. The RBTN facilitates the exchange of plant material, allowing breeders to evaluate varieties in comparison with those from other breeding programs, and evaluating the performance of varieties across the diversity of locations representing the cotton belt. Public regional yield trials are a valuable resource for examining genotype by environment interactions.

With the advent of newer technology to measure fiber quality with higher precision and accuracy, it would be valuable to test these METs samples and compare to current industry standard analysis. One recent study compared selection gains made using the two most widely used instruments for quantifying fiber quality: HVI and AFIS. Interpretation of fiber quality data was determined to be critical to making selection gains, but the study's conclusions were focused primarily on fiber length. The paper did not address the effect of line's selection based upon fiber micronaire, fiber fineness, or maturity ratio (Kelly et al., 2012).

The objective of this study was to compare eleven randomly selected genotypes for fineness (mtex) and maturity ratio when measured with the Cottonscope and HVI micronaire within one location. Examining this set of genotypes from a single location built a working knowledge of the instrument, confirmed previous information about the relationship between these traits as well as examined the potential advantages of using the Cottonscope to examine fiber fineness and maturity instead of relying solely on HVI data as a tool for breeding selection.

2.2 Materials and Methods

Eleven genotypes were selected randomly from the thirty-four entries harvested from the 2012 RBTN trial at Dean Lee Research Station in Alexandria, LA. The experimental design was a randomized complete block with four replications. Plant spacing was 0.97m (38 inches) between rows with a within row spacing of 0.1m (3 plants/ft.). The crop was managed according to LSU Agcenter Best Management

Practices at each site with regard to fertility, weed and insect control for a conventional (non-transgenic) cotton crop.

Fiber samples were obtained from twenty-five hand-harvested bolls, randomly harvested from two-50ft-row plots in all four replications. These samples were processed with a 10-saw laboratory gin to separate fibers from the seeds (Dennis Manufacturing, Athens, TX). Because of the hygroscopic nature of cotton fibers, temperatures and RH significantly affect fineness measurements (Rodgers et al., 2012a). Therefore, samples were equilibrated to standard environmental conditions. For forty-eight (48) hours prior to fiber quality measurement, fiber samples were held at a temperature of 70 ($\pm$) 1 °F and relative humidity of 65 ($\pm$) 2% (ASTM D1776/D1776M, 2015). Fiber samples were first tested with HVI with two 10g plugs per sample to quantify MIC by airflow resistance.

The fiber sample's fineness and maturity ratio were quantified per the protocol defined by previous work comparing the Cottonscope values to image analysis microscopy (Rodgers et al., 2012a). Each genotype was quantified with 6 data points per field replicated plot for their fineness and MR values.

2.3 Results

All genotypes had one MIC measurement at the discount value of 5.0 (Fig 2.3.1). When looking at entry 13, the average micronaire value (Fig. 2.3.1) is within the base range and is similar to many other genotypes. However, when the fineness (Fig. 2.3.2) and maturity ratio (Fig. 2.3.3) values are examined, the difference from other genotypes is evident. Comparing across entries, it is evident that the relationship between MIC is stronger with fineness than maturity. Also, the Cottonscope detected wider range of fineness and maturity ratio than HVI could detect in MIC.

Entries 2 and 34 have the highest mean MIC value and, as traditionally thought, are the coarsest fiber samples (highest mtex). Entry 28, however, has a relatively close MIC yet is a much finer fiber (lower mtex). The maturity ratio in these samples demonstrates the perplexing nature of the fiber traits

13

relationship. Entry 28 and 34 are high maturity fibers and entry 2 is one of the lowest. All samples had

maturity levels above what is considered low (<0.80). Many genotypes are not significantly different

when examining MIC but are significantly different in both fineness and maturity.
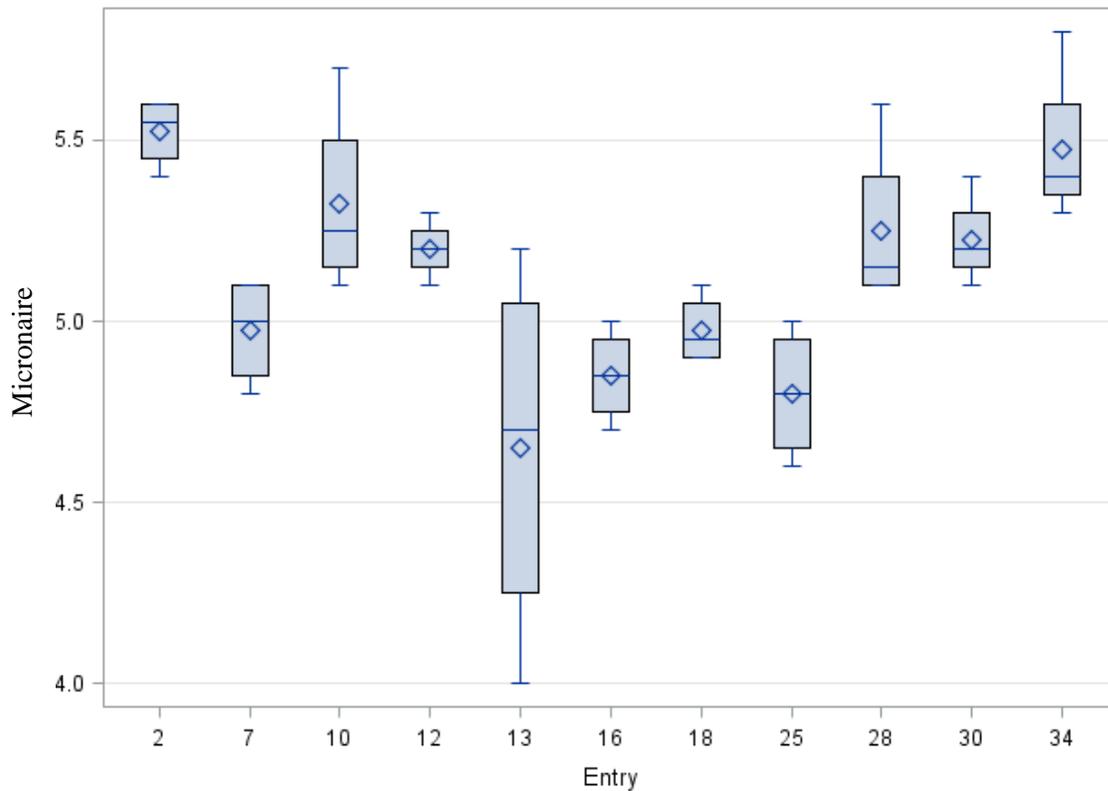

Figure 2.3.1. Distribution of fiber micronaire from eleven random entries sampled in Alexandria, LA of
the 2012 Regional Breeder's Testing Network

Correlation (Pearson's r) analysis of HVI micronaire and Cottonscope fineness, maturity,

micronaire (calculated from fineness and maturity), and area reveals (Table 2.3.1) very strong correlations

exist between these measurements.

Figure 2.3.2. Distribution of fiber fineness (mtex) from eleven random entries sampled in Alexandria, LA of the 2012 Regional Breeder's Testing Network

Fiber fineness showed very strong correlations with both Cottonscope and HVI micronaire as well as fiber area. The correlation of fineness and maturity was essentially non-existent. Fiber maturity was correlated (r=0.42) with Cottonscope micronaire but no relationship with HVI MIC. HVI micronaire had a very strong relationship with fiber fineness (r=0.76) and a strong relationship with Cottonscope micronaire (r=0.64) and fiber area (r=0.46). These results indicate that HVI micronaire correlates well with fiber fineness and very little with maturity.
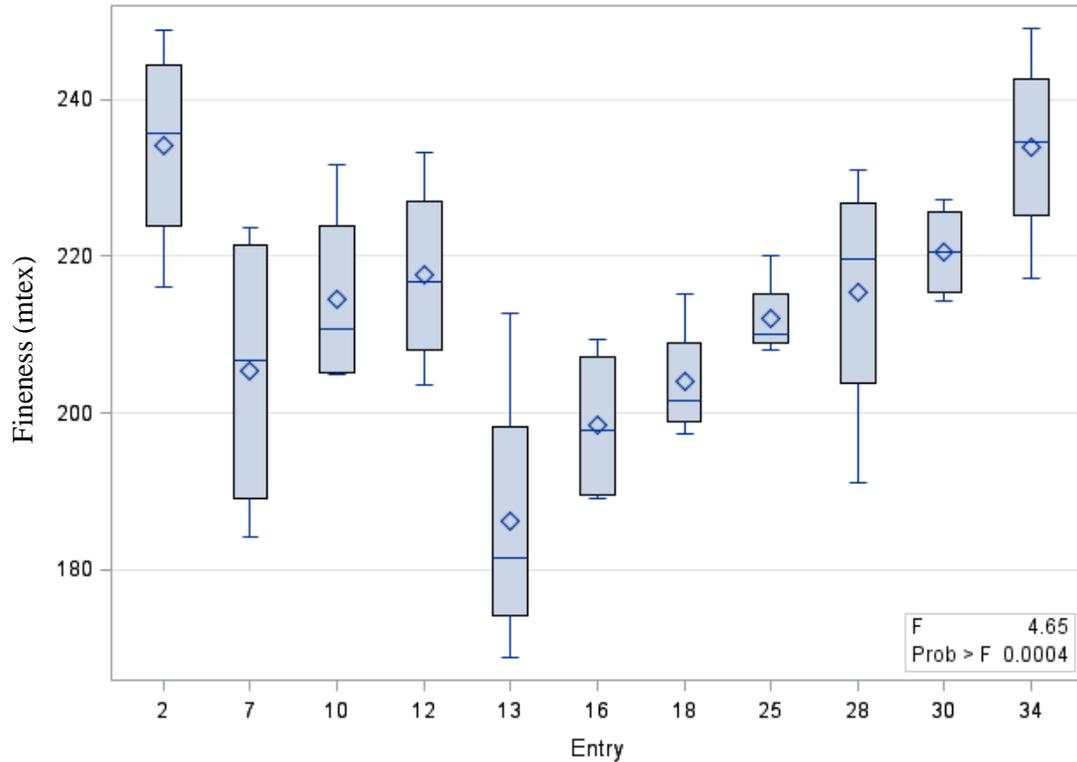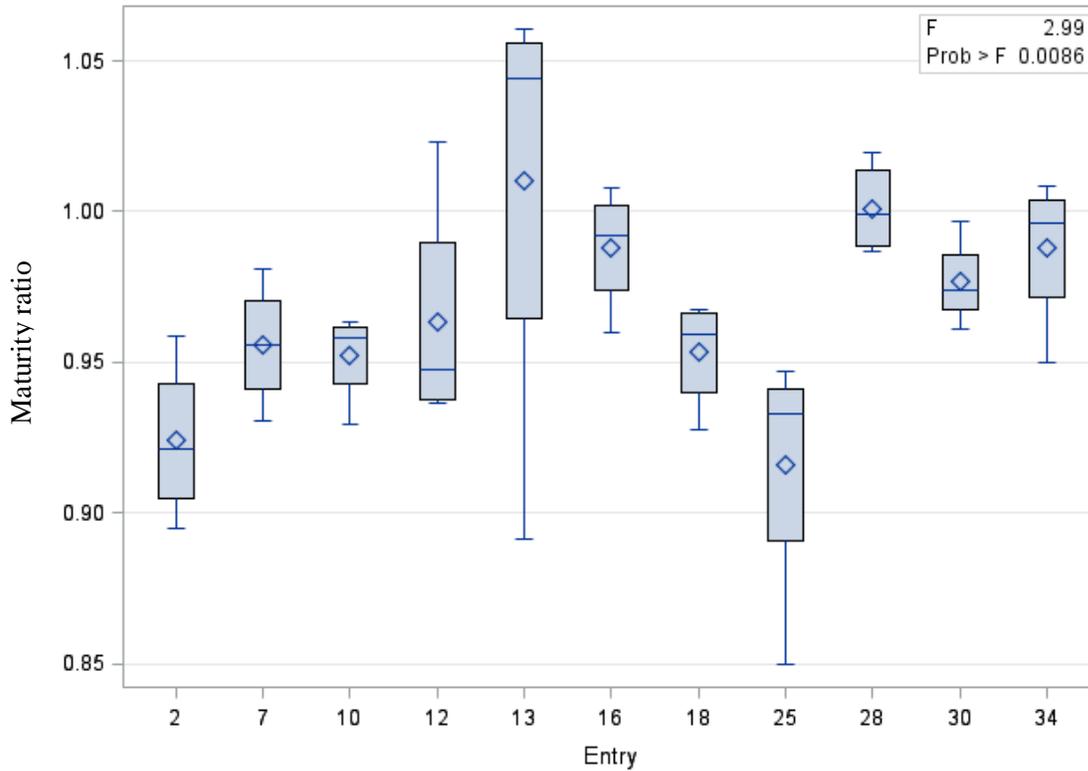
Figure 2.3.3. Distribution of fiber maturity ratio from eleven random entries sampled in Alexandria, LA of the 2012 Regional Breeder's Testing Network

Table 2.3.1. Correlation analysis of Cottonscope and HVI quality data from eleven random entries sampled in Alexandria, LA of the 2012 Regional Breeder's Testing Network

	MR	Fine	CS MIC	Area	HVI MIC
MR	1.00				
Fine	-0.02	1.00			
CS MIC	0.42*	0.78**	1.00		
Area	-0.17	0.74**	0.75**	1.00	
HVI MIC	0.12	0.76**	0.64*	0.46*	1.00

*Statistically significant at $p<0.05$.
**Statistically significant at $p<0.01$.

2.4 Discussion

  Significant differences between genotypes are not always present when examining micronaire.

Due to the limitations in using airflow resistance to indicate fineness and MR values, it appears that a type

II error occurs, investigations fail to reject the null hypothesis when a significant difference is truly present. While discrimination between samples using MIC is not always possible, the Cottonscope does detect significant differences between the component traits. Interaction between fineness and MR are a likely cause of similar micronaire values, but because the purpose of using micronaire is to indicate relative fineness and MR of samples these results show how micronaire is insufficient. Samples exhibiting equivalent micronaire values do not always have equivalent fineness and maturity values.

Numerous studies have found no significant differences between treatments when examining MIC. In light of the results of this study, significant differences in fineness or MR can be present but undetectable using HVI MIC. These undetectable differences using MIC may have a significant impact on spinning performance and final yarn quality which could be estimated using higher precision instrumentation. The results of this study warrant using the Cottonscope to detect variation in fineness and MR for breeding especially where sample size is limited.

Micronaire readings do not represent distributions within samples. Only instruments that analyze individual fibers or fiber sections can capture how variable a genotype is within an environment. This study demonstrates the need for instrumentation that measures fineness and maturity directly. MIC is a poor representation of these two. Without a measure of fineness or maturity it is ineffective to study MIC or make genetic improvements. Applying direction selection for micronaire will be complicated by the interaction between fiber fineness and maturity.

The uniformity across genotypes is not consistent. Several varieties displayed wider distributions than others in their fiber quality. Genotypes which exhibit a wider distribution of fiber quality parameters are less desirable because the increased variation makes spinning efficiency inconsistent and more difficult to predict. Considering the amount of variability demonstrated in this sample of genotypes within an environment, it begs the question: what are the significant within environment sources of variation?

This investigation supports the need for more accurate and precise measurements of fineness and maturity when trying to improve fiber quality. Micronaire does not adequately distinguish between samples to the degree needed for breeding selection or other scientific research. The varieties used were genetically diverse yet the differences present in either fineness or MR were unperceivable when looking at MIC. It is likely that within a segregating population, differences remain undetected when using MIC. Whether the research goal is to test a treatment effect or select for fineness or MR, using MIC appears to be inadequate. Independent measurement of these fiber parameters may allow for successful selection of improved genotypes and increase the power of investigations as to how various treatments influence fiber parameters.

Chapter 3 - Yield of upland cotton genotypes by fruiting position

3.1 Introduction

Upland cotton (*Gossypium hirsutum,* L.) natively is a perineal shrub with an indeterminate growth habit. Through domestication is has been adapted to annual cropping systems across the globe. The plant's indeterminate nature means that it produces both vegetative and reproductive biomass at the same time. The management practices, specifically row spacing and plant density, typical of production in the United States, tend to produce plants with a single monopodium. From this main stem, fruiting branches extend outward developing flowers that potentially set cotton bolls. Where spacing allows, side branches can form to compensate for gaps. The side-branches also have the capacity to extend fruiting branches however the yield formed on these branches represent only a minor portion of total yield when population density is managed properly (Jenkins et al., 1990).

Cotton is grown primarily for the value of its fibers. Cotton plants flower continuously and periodically throughout the growing season. Successfully fertilized flowers develop into the harvestable component of cotton yield referred to as bolls. Cotton fibers are the dried remnants of thousands of cells extending from the epidermis each seed. Each fiber is a single cell which has undergone a length period development categorized by two distinct phases: 1) cell wall elongation, and 2) secondary cell wall development. It takes as many as 60 days for a flower to mature into an open boll (Oosterhuis, 1990).

Cotton's indeterminate growth habit results in physically and temporally spaced boll development. Over an approximately 60 day period of boll development and maturation, plant yield components (seed and fiber quantity) are subject to a diversity of environmental conditions. As with many agricultural traits, genotypic differences and their interaction with environment (GEI) also contribute to variation. This is typically investigated at a whole plant level but in cotton, given the length of the boll development and maturation period, it is hypothesize that yield components within a genotype will display significant variation as a result of fruiting site (vertical node and horizontal position).

Cotton plants set flowers in a predictable pattern. Flowers are set at the same sympodial position three (3) days apart for each internode between them. Bolls along a single sympodium are six (6) days apart for each change in position (McClelland, 1916; Oosterhuis, 1990). For example: the first flower sets on position one, and three days, later the first position boll on the next vertical node sets. Six days after the first position flower sets, position two will set a flower. This pattern is a reliable for estimating relative differences in boll ages. Lower bolls remain on a plant longer than upper bolls, and inner (first position) bolls spend more time on a plant than outer bolls. Lower, inner bolls have more time and resources with which to develop mature fibers.

Short-season cotton genotypes can exploit the purported advantages in early maturity and plant breeders have made selection for such cotton genotypes. Buie (1928) reported advantages in early crop maturity as a means to circumvent damage caused by the boll weevil (*Anthonomus grandis,* B.) epidemic. Prior to the wide adoption of *Bacillus thuringiensis* (Bt) transgenic technology, managing crop maturity was considered a viable option for reducing damage caused by insects such as the cotton budworm (*Heliothis virescens,* F.) and the bollworm (*Heliocopcerpa zea,* B.) (Heilman et al., 1979; Namken and Heilman, 1973; Shepard, 1982). By reducing the amount of time needed to reach crop maturity, the potential for insect-damaged plants and insecticide costs were reduced. Heilman et al. (1979) found that short-season genotypes cost less to grow and yielded more. Other work did not find a significant reduction in insect populations or insecticide use, but the advantage of early maturing genotypes was that they allow for a one pass harvest (Roach and Culp, 1984). Breeders continue to develop and market early-maturing varieties for management and climatic reasons.

Full-season varieties have more recently come into favor in the Southern states where a longer growing season is available. It is considered that full season types buffer yield against unfavorable abiotic and/or biotic conditions which may occur during flowering and boll maturation. Full-season varieties are known to be better adapted to recovering and compensating for aborted flowers resulting from drought (Bednarz and Nichols, 2005). Modern full-season varieties may be better suited at buffering against yield

20

loss but the longer growing season translates to a wider range of environmental variation subjected to each developing boll.

When utilizing full-season varieties, management decisions by producers extend beyond plant spacing and fertilization. Plant growth regulators (PGRs) are used to manipulate plant growth. The rate and timing of applications is critical to achieve the desired effect. As plant growth regulators became more popular tools in a producer's arsenal, changes in crop management responded accordingly. PGRs are used for a multitude of effects on the crop's physiology such as reducing internode elongation, stimulating defoliation, and expediting boll opening (Reddy et al., 1990; Zhao and Oosterhuis, 2000). An array of chemicals are used to achieve shorter plants with less vegetative biomass, improved boll load, and a rapid defoliation (Oosterhuis and Robertson, 2000). Their ability to increase yield historically has not proven consistent (Oosterhuis et al., 1991). While yield increases upon PGR treatments have been reported (Gencsoylu, 2009), O'Berry et al. (2009) saw a reduced yield under Pix™ (mepiquat chloride) treatment. Despite this, PGRs are profitable because they reduce the cost of harvesting and increase the fiber value through a decrease in trash. For these reasons, applications of PGRs have become routine for cotton production, especially in areas growing indeterminate plant types.

Environmental factors have a dramatic effect on per acre yield of cotton making selection gains a slow process. On a large scale, this phenomenon has been well established, and GEI are the subject of considerable research. Studied to a lesser extent, is how yield distribution varies within genotypes. In prior research, yield of fruiting sites has been quantified showing that first position bolls comprise 66-75%, and second position bolls comprise 18-21% of total yield in the cultivars examined (Jenkins et al, 1990). This study demonstrated a change in fruiting site retention between older cultivars (1962) and newer (1986) early-maturing cultivars. The change in boll distribution was found to be significant, and Jenkins et al. (1990) noted that this information could impact management decisions.

Just as environment can affect the yield of whole plants, each boll on a cotton plant is subject to its own micro-environment due to the temporal way in which bolls mature both vertically and horizontally. Abiotic factors can affect both fruit retention but also fiber properties since these are developed over an approximately 60 day period. Physiological competition for resources within the plant can also affect both fruit retention and fiber properties. Considered collectively, a plant's fruiting pattern is determined by the interaction between genotype and both gross and micro-environment. From this arises the question of whether different fruiting patterns may be more or less stable to environmental changes and whether fiber properties within a plant may be more uniform (stable) or variable (unstable).

Uniformity and stability are important to all cotton stakeholders. Breeders work to develop varieties that can perform as expected across years in their target environments. Producers expect varieties to yield consistently when inputs are managed properly. Agronomists and physiologists depend on uniformity of cultivars when treatments are applied. Variation, however, is crucial to survival, adaption, and evolution. Therefore, stakeholders are bound within the limits of natural variation to meet their expectations for uniformity and stability. Understanding the sources of within-plant variation will improve the ability to develop stable varieties.

Previous studies have looked at yield and fiber quality within a cotton genotype's architecture and found significant differences between individual fruiting positions (Jenkins et al, 1990a; Jenkins et al, 1990b; Conkerton et al, 1993; Davidonis et al, 2004; Ritchie et al, 2014; Zhao et al., 2012) One study failed to find a significant difference in lint yield when bulking fruiting positions by plant zones, based on assumed flowering dates. However, lint percent, seed weight, fiber quality were significantly different between plant zones (Hague et al., 2014).

One factor that is considered in this type of analysis is the effect of position alone on boll maturation. First position bolls are likely to have more access to draw nutrients and photosynthate than second or third position ones. The majority of lint is produced by first position bolls at lower nodes.

Examining within-genotype yield distribution will identify how yield is partitioned in Louisiana adapted lines. Unique yield distributions may require unique management decisions to effectively target the major portion of crop yield. Jenkins et al. (1990) found that first, second, and third position bolls represented 71, 20, and 3% of yield respectively. Changes in variety characteristics over the past 25 years may have resulted in significant deviation from that yield pattern which, consequently, would require a different management approach.

Variation in cotton yield from bolls within a plant has both positive and negative consequences. Physiological responses to environment can allow a crop to adapt, in season, to unusual conditions so that yield is not stunted. Likewise, differences between genotypes for the variation they exhibit within a plant indicate an opportunity for genetic selection. Genotypes can be bred that are stable yielding in stressful environments while also being good performers under ideal conditions. The environment can only be controlled in part, thus it is valuable to have varieties which can buffer uncontrolled aspects of the environment.

Using varieties specifically adapted to a target production region offers better, more stable yield and fiber quality. The purpose of this study is to better understand the within-plant variation of fiber yield of three Louisiana adapted varieties (LA10307140, LA10307108, and LA10307021) and two control varieties (FM958 and DP393) in two Louisiana environments over a two year study.

3.2 Materials and Methods

In 2013 and 2014, five cotton genotypes were grown in the field in two-row plots in both Alexandria and St. Joseph, LA. The experimental design was a randomized complete block with four replications. Plant spacing was 0.97m (38 inches) between rows with a within row spacing of 0.1m (3 plant/ft.). The crop was managed according to LSU AgCenter Best Management Practices at each site with regard to fertility, weed and insect control for a conventional (non-transgenic) cotton crop. Three genotypes were unreleased adapted genotypes developed by the Louisiana State University cotton breeding program, and two were commercially developed conventional cotton varieties. The LA varieties

(Table 3.2.1) were selected due to their diverse pedigree and successful performance in internal yield

trials. The two commercial varieties were selected for this study due to their stable, high yielding

performance checks in regional trials.

Table 3.2.1. Five genotypes examined for within-plant yield variation in Alexandria, LA and St. Joseph, LA in 2013 and 2014

LA adapted lines	Control lines
LA10307140	DP393
LA10307108	FM958
LA10307021	

Two weeks after defoliation, five plants were randomly selected within each plot to map boll

distribution. Plants were clipped at the soil surface and the cotyledonary node (0) used as a reference

point. Bolls were hand-harvested and combined within each field replication according to their fruiting

positions (Jenkins, 1990a and 1990b, and Conkerton et al., 1993). Boll count by fruiting site was recorded

as plants were harvested. This process was repeated across both years and both locations for each

genotype.

Total seed cotton weight within each position was recorded before the samples were processed

using a 10-saw laboratory gin (Dennis Manufacturing, Athens, TX). Lint and seed weights were recorded

in grams. This data was used to calculate the following yield components at the individual fruiting

positions: seed cotton weight (g), lint weight (g), lint % (100*lint wt./seed cotton wt.), seed % (100*seed

wt./seed cotton wt.), total boll count of sampled plants, average number of effective fruiting sites (boll

count/five plants), node to first boll, and node to last boll.

Genotypes are treated as fixed effects. Node and fruiting position were treated as fixed effect

factors because flowering occurs continuously and each boll is subjected to an array of environmental

conditions. The other factors to evaluate in this study are the year effect and the location effect and both

were considered as random effects. PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC) was used to calculate an Analysis of Variance (ANOVA) for determining treatment differences. PROC UNIVARIATE was used to generate histograms and calculate Goodness-of-Fit for the distributions.

3.3 Results

The results of this study show the differences in fruiting pattern for five genotypes. Table 3.3.1 summarizes the genotypes across years and locations to illustrate their basic fruiting behavior. Within this study, the first boll was found at node 5.5-6.8 and the last boll was at node 19.5-21.3. The range of fruiting nodes ranged from 13.8-15.5. LA10307108's boll weight was the lowest, but with the widest range of fruiting nodes and the largest number of bolls this line had the greatest total weight. LA10307021 began fruiting slightly later (node 6.8) and had a smaller range of fruiting nodes, but had the largest average boll weight and was the second highest yielding.

Table 3.3.1. Summary of yield components for five genotypes grown in Alexandria, LA and St. Joseph, LA in 2013 and 2014

Genotype	Lowest Node	Highest Node	Fruiting Range	Number of bolls	Total weight (g)	Boll weight (g)
DP393	5.8	19.5	13.8	27.9 ± 4.0	274.9 ± 5.4	4.6 ± 1.3
FM958	6.0	21.3	15.3	29.1 ± 3.5	285.8 ± 5.9	4.6 ± 1.3
LA10307140	6.0	20.0	14.0	25.5 ± 9.5	267.4 ± 6.3	4.6 ± 1.3
LA10307108	5.5	21.0	15.5	30.3 ± 8.7	294.3 ± 6.5	4.2 ± 1.1
LA10307021	6.8	20.8	14.0	26.9 ± 2.5	290.5 ± 6.4	4.7 ± 1.2

Pearson correlation analysis between yield components indicates which traits are most strongly associated with total yield of the sampled plants (Table 3.3.2).The strongest correlation between traits was between total weight (5 plants sampled) and both average weight per plant and lint weight per plot (r=0.95). This was expected considering the direct relationship between seed cotton and lint weight. The relationship between seed cotton weight per plot and number of effective bolls was also very strong

(r=0.82). Strong correlations were present between several important yield components. A strong negative correlation with average boll weight and number of bolls per plant (r=-0.595) was detected.

Table 3.3.2. Pearson correlation analysis of yield components of five box mapped genotypes grown in Alexandria, LA and St. Joseph, LA in 2013 and 2014

	1	*2*	*3*	*4*	*5*	*6*
1. Number of bolls per plant	1.00					
2. Total seedcotton weight (g)	0.82**	1.00				
3. Boll weight (g)	-0.59*	-0.06	1.00			
4. Weight(g) per plant	0.76**	0.95**	0.06	1.00		
5. Lint percent	0.15	0.22	-0.02	0.15	1.00	
6. Total lint weight (g)	0.76**	0.95**	-0.06	0.87**	0.49*	1.00

*Statistically significant at p<0.05.
**Statistically significant at p<0.01

The Analysis of Variance (Table 3.3.3) showed the amount of variance attributable to each factor on the yield components. Node, position, and node*position were highly significant effects on total lint weight of the sampled plants. Similarly, node, position, and node*position were highly significant effects on boll count. Genotype*position was also a significant effect on boll count.

Average boll weight was calculated by dividing total weight bulked per fruiting site by the corresponding boll count. Variance in average boll weight was influenced significantly by genotype and node*position. No interactions between these sources were significant.

Lint percent exhibited very little variation within this experiment. No factor, fixed or random, had any significant effect on lint percent. Based on this extremely narrow variation and the direct relationship between total yield, lint % (lint yield/total yield*100), lint yield (total yield*lint%), and seed yield (total yield-lint yield), the trends of these traits are the same. Only distribution of lint yield was illustrated but the relationship between total yield, lint yield, and seed yield means that trends exhibited in one trait are also exhibited by the others.

Table 3.3.3. Analysis of variance for fineness (mtex), maturity ratio, and Fibronaire micronaire from five genotypes grown in Alexandria, LA and St. Joseph, LA in 2013 and 2014 sampled by fruiting site (node by position) of bolls

Source	DF	Lint weight (g) MS	Boll count MS	Average boll weight (g) MS	Lint percent MS
Genotype	4	22.42	0.18	6.14**	0.003
Node	20	231.62***	5.98***	8.04***	0.025
Genotype*Node	66	21.06	0.88	1.50	0.023
Position	2	1960.95***	50.68***	39.14***	0.040
Genotype*Position	8	44.38	1.94*	0.91	0.024
Node*Position	28	51.65***	1.69***	1.88	0.033
Genotype*Node*Position	86	15.95	0.55	1.44	0.044
Year	1	11.32	8.54	20.08	0.124
Location	1	24.65	2.41	2.39	0.197
Year*Location	1	176.45*	2.69	1.47	0.003
rep(Year*Location*Genotype)	32	42.34**	1.31*	2.21*	0.053
Residual	867	23.15	0.88	1.38	0.063

*Statistically significant at p<0.05.
**Statistically significant at p<0.01

Due to the significant effect of node, position, and their interaction on total lint yield, histograms were generated to show the distribution of yield by each of these factors. Each bar represents the percent of yield found at each position (Fig 3.3.1) and node (Fig 3.3.2). Fig 3.3.1 show that regardless of genotype, the majority of lint yield is upon first position bolls, followed by second then third position bolls. Data was combined across years and locations because they lacked a significant effect on the variation present.

Distribution of each genotype's total lint yield (g) represented as a percent by position demonstrates the value of first position bolls. With all genotypes, more than 50% of lint yield was found on first position bolls. Second position bolls, represented 20-30% of lint yield. Third position bolls of any genotype were less than 15% of the yield. LA10307140 had the largest yield on first position bolls and the lowest yield held on third positon. Within each genotype, lint yield by position does not follow any standard distribution curves. All goodness-of-fit statistics indicated that normal, lognormal, gamma, beta, or Weibull distributions were not adequately fit based on Anderson-Darling criteria.
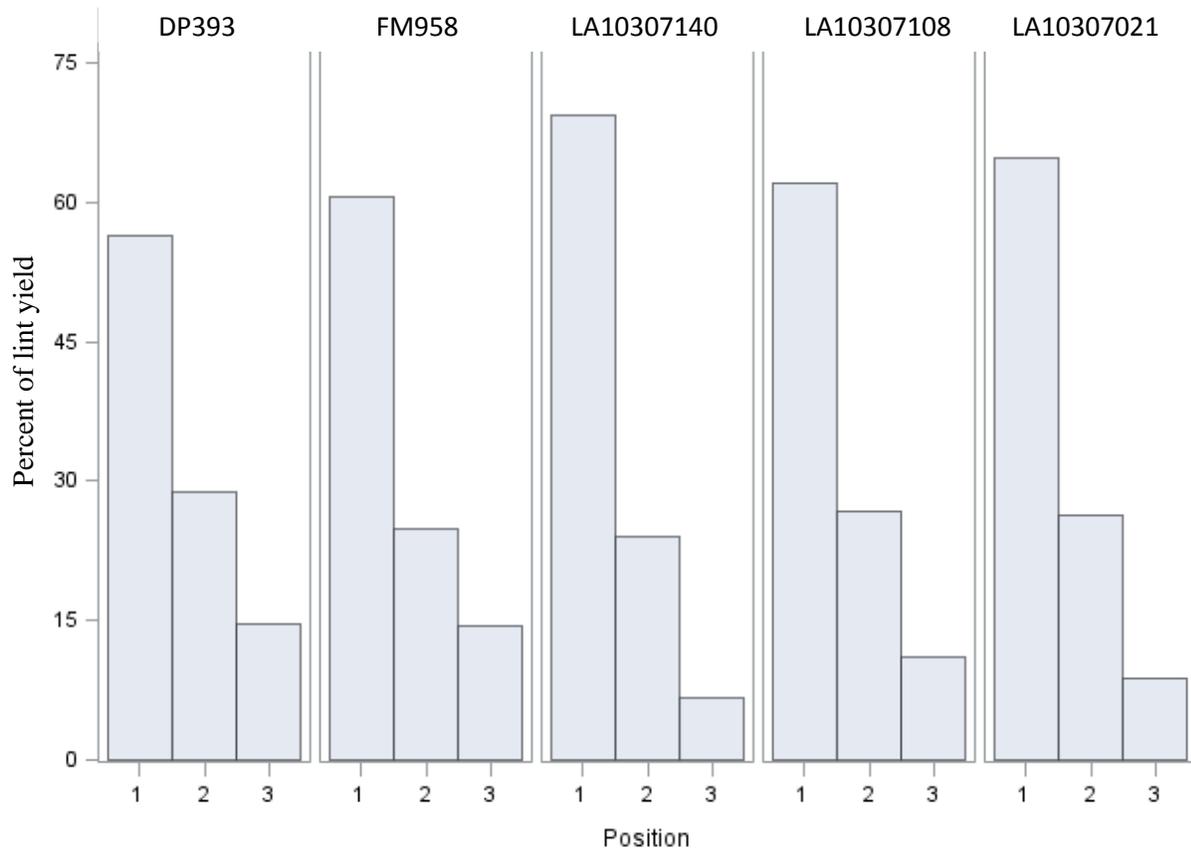
27

Fig 3.3.1. Percent of lint yield by position of five box-mapped genotypes grown in Alexandria, LA and St. Joseph, LA in 2013 and 2014

The distribution of total lint yield at each node (x-axis) as a percent of the total lint yield (y-axis) within a genotype is presented in Fig 3.3.2. The mean fruiting node was similar for all genotypes and ranged from 11.356-12.384. The genotypes differed in the way yield was distributed about their "mean" node. All within genotype distributions displayed a positive skew. There were more nodes above the mean node, however their individual contribution to total lint yield was less than those below node twelve. This information compliments the trend that average boll weight decreases as bolls set higher up on the main stem of the plant. The distributions of lint yield within any genotype did not fit normal, Weibull, or gamma curves based on Anderson-Darling criteria.
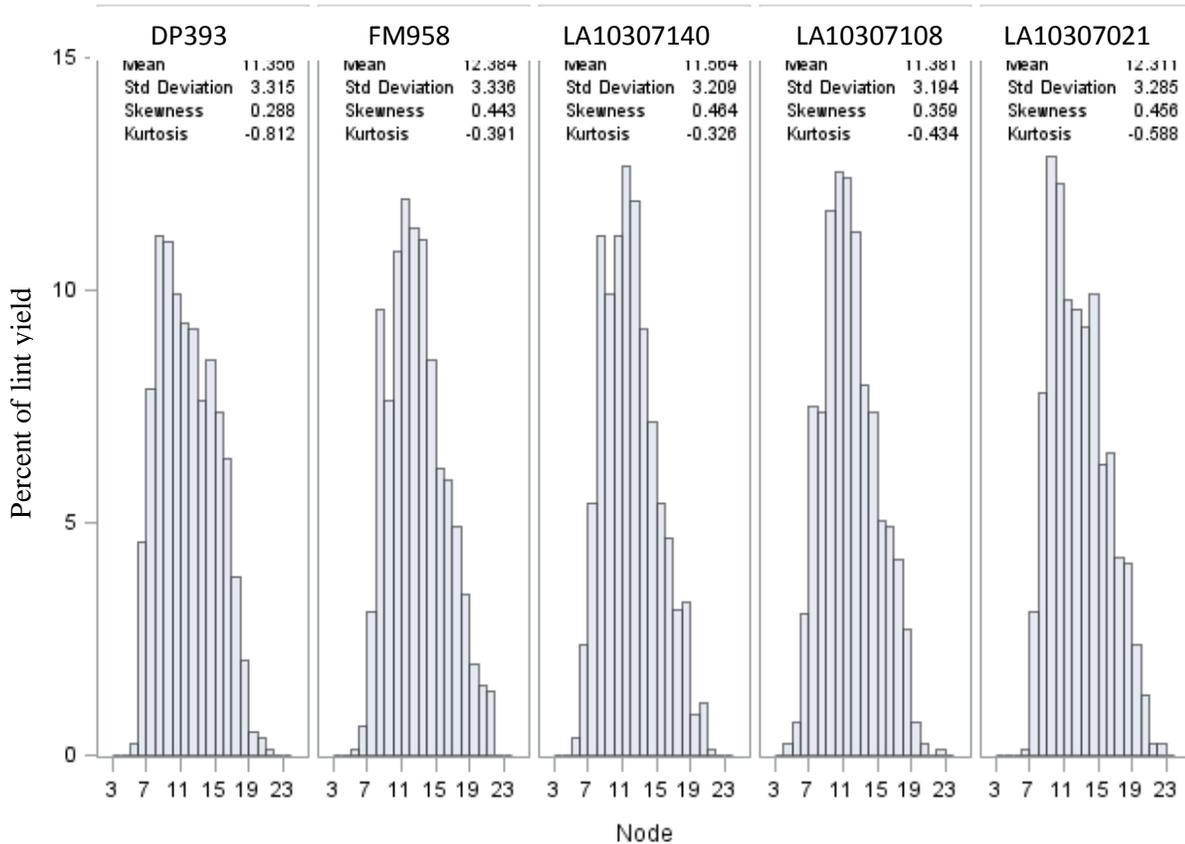
Fig 3.3.2. Percent of yield by node within five box-mapped genotypes grown in Alexandria, LA and St. Joseph, LA in 2013 and 2014

The distribution of the lint yield within genotypes by Node and Position (Fig 3.3.3) shows the node*position interaction existing within genotypes. The X-axis represents the main stem nodes; the Y-axis represents percent of yield within each horizontal position. This comparative histogram illustrates further the difference in yield distribution between and within cotton genotypes. Across all genotypes, first position bolls have a higher mean yield, a wider range of effective nodes (6-21), and a more consistent distribution of yield between nodes. At position two, the range of nodes decreases (6-18). Position three bolls are typically found at a lower range of nodes (6-15). The decrease in the range of nodes holding a second and third position boll is one explanation for the decreases in yield from first to second to third position.
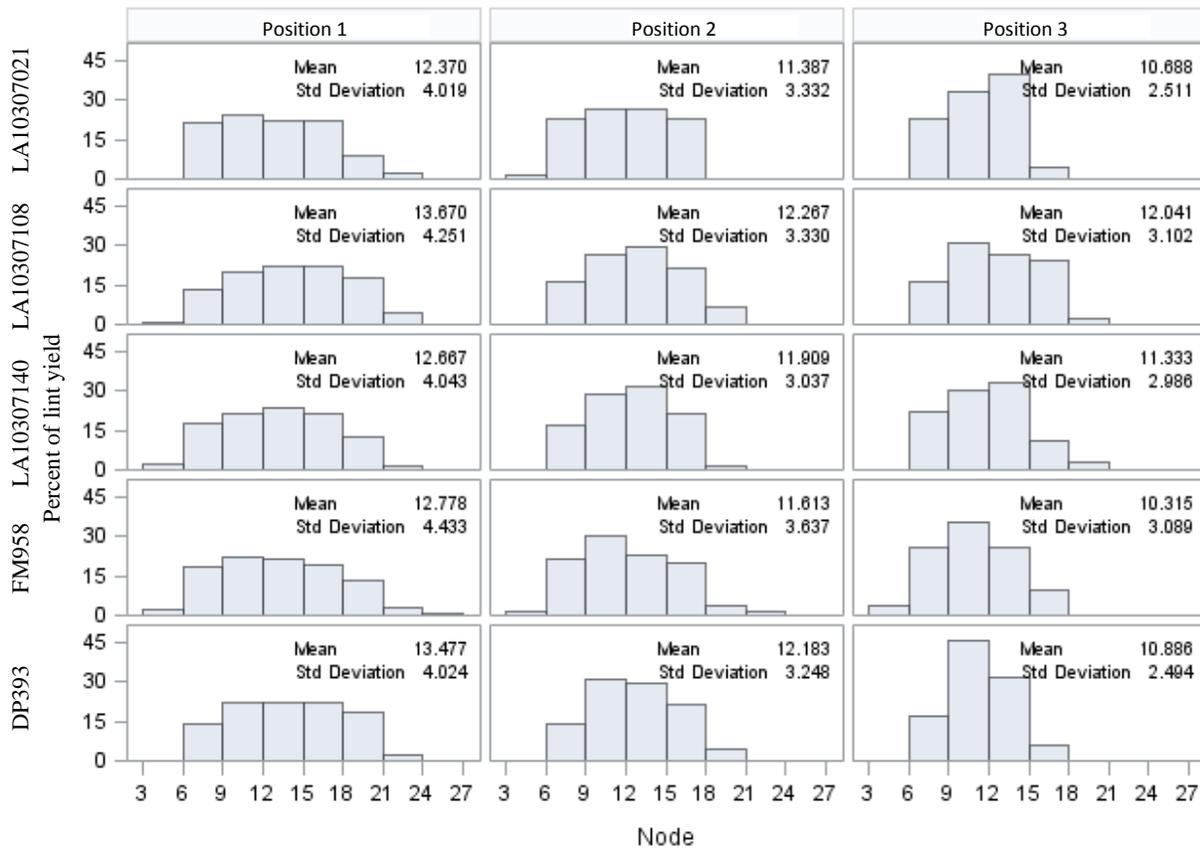
Fig 3.3.3. Percent of yield by nodes and positions within five box-mapped genotypes grown in Alexandria, LA and St. Joseph, LA in 2013 and 2014

A second reason for the decrease in lint yield as position moves outward is the decreased boll weight. First position bolls were heavier (4.601-5.004g) than second position bolls (4.041-4.507) which were heavier than third position bolls (3.731-4.446). The comparative histogram (Fig 3.3.4) represents the distribution of average boll weight produced within each horizontal position. With the exception of genotype FM958, a trend exists where the mean boll weight within a position decreases as bolls set farther out from the main stem. Also, the skewness values shift to less-negative values as bolls set further from the main stem.
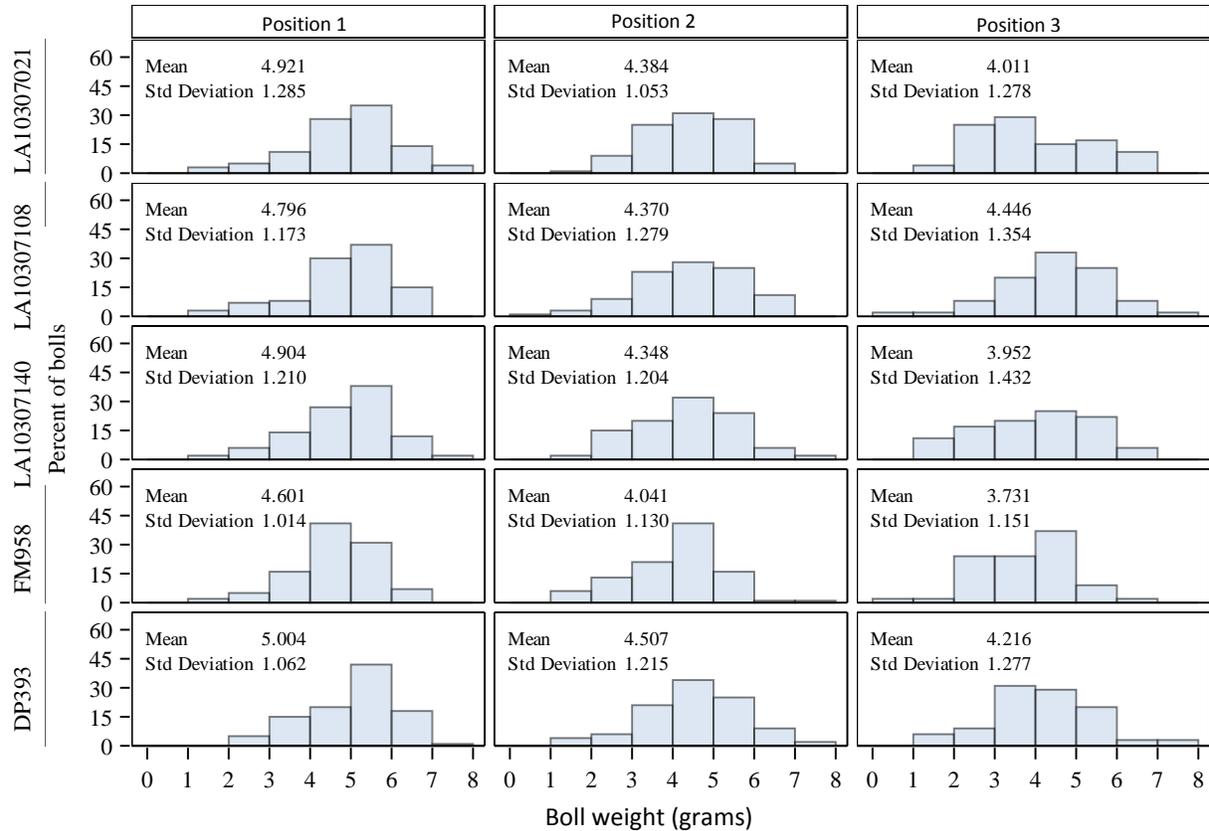
Fig 3.3.4. Average boll weight by positions within five box-mapped genotypes grown in Alexandria, LA and St. Joseph, LA in 2013 and 2014

3.4 Discussion

Boll weight decreased significantly as boll position increased. This trend is consistent with the findings of Jenkins et al. (1990), however the percentage of yield at each position was different. Jenkins et al. (1990) reported 71, 20, 3% at first, second, and third position bolls. In this study, there was a lower percentage of lint yield at position 1 (60.7%) and a greater percentage of lint yield found at position 2 (27.0%) and position 3 (12.3%). Environmental differences between the studies could account for some of this difference, however genetic selection for adapted lines and the tools of modern management practices allow cotton crops to hold and support more of the later position bolls than was possible in 1990.

Our results for lint % variation differ from that reported by Hague et al. (2014) where bolls were combined into three fruiting zones based on estimated boll age. That study found genotypes to be a significant source a variance for lint % possibly due to a larger set of genotypes tested. However, lint %

31

was often higher in the upper fruiting zone (Hague et al., 2014). In our study, lint % had an extremely narrow variance across all genotypes, fruiting sites, and environments tested. The lack of significant variation in our study indicates that the genetic variation of these inbred lines is fixed and that interaction with environment is not a significant source of variation. Lint % has been reported as the most heritable (h^2=97%) of all the yield components (Desalegn, 2009). The high reported heritability and the findings in this study indicate that selection in segregating populations for lint % is very effective and the resulting lines will be stable across environments.

Ulloa (2006) found that yield components (boll weight, lint %, number of bolls) are significantly correlated and most often in ways that present problems for breeding selection. Improvement of one yield component negatively affects a different component. In this study, boll weight and number of bolls per plant were negatively correlated with each other as found in other studies: Miller et al. (1958), Bridge et al. (1971), and Desalegn (2009).

These findings differ from Desalegn (2009) where 1) lint yield was significantly correlated with boll weight and bolls per plant, and 2) lint % was positively correlated with lint yield, boll weight, and bolls per plant. In this study, total lint weight was positively correlated with number of bolls, but no correlation between boll weight and total lint weight was detected. Also, lint % exhibited no significant variation and no correlation to the other yield components.

A strong correlation was present that would suggest boll weights decrease with an increase in boll load. The source-to-sink dynamic compensates accumulation of individual boll weight with different boll load. These genotypes seem to be able to compensate for boll loss through an increased accumulation of resources in the remaining bolls. This compensation is likely beneficial if early season conditions do not favor boll set. Conversely, excessive late season bolls which lack time to fully mature is not favorable as they draw resources from other developing bolls.

A strong negative correlation (r=-0.59) exists between average boll weight and bolls per plant. This relationship demonstrates the source/sink dynamic present in a cotton plant. Bolls per plant, average boll weight, plot weight, and fiber maturity are all interrelated to a significant degree.

First position bolls were heavier and more numerous consequently representing the largest percent of yield. Inputs are better utilized if directed toward these bolls because they represent the majority of the crop yield. Lower, inner bolls experience a longer duration of solar exposure, higher humidity micro-environment, and primary access to resources from the main stem vascular tissue. This may explain why lower, first position bolls are heavier and more successfully retained than second or third position bolls. The reduction in range of nodes holding second and third position bolls (Fig 3.3.3) shows that lower nodes have the highest boll load at any position. Higher nodes are less likely to develop mature bolls. This is likely a direct result of flowering interval and the of lack heat units in late season which are required for bolls to reach maturity.

Years and locations were not found to be significant for any of the traits examined. These traits were more affected by the boll's fruiting site. According to this data, there was more variation in these yield components by node, position, and node*position than due to the different years or locations tested. Lint yield varied more as a result of fruiting location than genotype. This suggests that lint yield is more affected by plant architecture within an environment than the influence of year and location. Plant architecture, while not visually different during active growth was noticeably different in boll load after defoliation and upon harvest. Correlation between specific fruiting patterns and total yield existed. Simply stated, lint yield was very strongly correlated with number of bolls per plant and the lint weight per plant. Enumerating bolls per plant or plot would be a valuable yield component for selecting genotypes. According to this data, breeding goals should focus on maximizing boll numbers per plant as a means to increase yield. In a full-season climate this may be achieved by selecting for plants with shorter flowering intervals (Bednarz and Nichols, 2005). A strong understanding of yield distribution and its role

33

in total yield and yield stability can direct breeding goals for maximizing efficient selection to increase

yield per acre.

Chapter 4 - Effect of Fruiting Position on Cotton Fiber Maturity and Fineness

4.1 Introduction

Cotton (primarily *Gossypium hirsutum* L. and to a lesser extent *G. barbadense*, *G. arboretum*, and *G. herbacium*) is the most important natural fiber crop in the world; the total economic revenue generated in the United States is over $100 billion. According to the United States Department of Agriculture's "Cotton: World Markets and Trade" report, the U.S. produced 15,000,000 bales of cotton in the 2013-2014 growing season, with only 3,700,000 of the bales being used domestically. The crop was produced on 3.98 million hectares of farm land (Cotton: World Markets and Trade, 2014). Cotton fiber is sold by weight and the price per bale is determined by its fiber quality profile. The "A" index, which represents the lowest international quotes, averaged 93.29 cents/pound between January-July in 2014, whereas the price received by farmers averaged 79.18 cents/pound (Monthly Prices, 2014).

Cotton's growth habit results in physically and temporally spaced boll development. Over an approximately 60 day period of boll development and maturation, plant yield determinants (seed and fiber quantity) are subject to a diversity of environmental conditions. As with many agricultural traits, genotypic differences and their interaction with environment (GEI) also contribute to variation. This is typically investigated at a whole plant level but in cotton, given the length of the boll development and maturation period, it is hypothesized that fiber quality within a genotype will display significant variation as a result of fruiting site (vertical node and horizontal position). Fiber quality is an important determinate in the pricing of cotton.

Cotton plants set flowers in a predictable pattern. Flowers are set at the same sympodial position three (3) days apart for each internode between them. Bolls along a single sympodium are six (6) days apart for each change in position (McClelland, 1916; Oosterhuis, 1990). For example: the first flower sets on position one, and three days later, the first position boll on the next vertical node sets. Six days after the first position flower sets, position two will set a flower. This pattern is a reliable for estimating relative differences in boll ages. Knowledge of this pattern leads one to recognize that lower bolls remain on a plant longer than upper bolls, and inner (first position) bolls spend more time on a plant than outer

bolls. In theory, lower inner bolls have more time and resources to develop mature fibers. The interval between flowers equates to a difference of 20 days or more between the first and last flower to develop a boll.

Cotton plants flower continuously and periodically throughout the growing season. Successfully fertilized flowers develop into the harvestable component of cotton yield referred to as bolls. Cotton fibers are the remnants of thousands of dried cell walls extending from the epidermis each seed. Each fiber is a single cell which has undergone a lengthy period development categorized by four distinct phases: 1) initation, 2) elongation, 3) secondary cell wall synthesis 4) drying. A fiber's physical properties are partially a result of genetic factors; however the influence of environment during each phase of development is a major source of variation. Less than ideal conditions during elongation will result in shorter fiber content. Similarly, less than ideal conditions during secondary cell wall deposition will increase immature fiber content. It takes more than 50 days for a flower to mature into an open boll (Oosterhuis, 1990). The long developmental window of individual bolls in combination with the temporal difference between bolls results in plants to developing fibers throughout a wide range of environmental variation. Consequently, the environmental variation will be reflected in the variation of fiber quality within plants.

Quantity per unit area in combination with quality, as a mean value determines a crop's economic return. Variation of cotton fiber quality within a crop is equally important but often overlooked because the tools available to quantify it are not widely available. A plant with more uniform fiber quality across fruiting sites would provide advantages over one more variable. Fiber quality parameters routinely measured include length, length uniformity, strength, elongation and micronaire. Of these, micronaire (MIC) is the parameter most affected by environment (Meredith et al., 2012). Micronaire is a unit-less measurement derived by measuring the airflow resistance of a cotton plug and roughly corresponds to fiber thickness. The components of micronaire are fiber fineness (mtex) and fiber maturity ratio (Table 4.1.1), and these interact in a complex way in relation to micronaire (Fig 4.1.1). Figure 4.1.2 gives a

representation of the ideal. Fiber fineness and maturity ratio were, until recently, both laborious and time consuming to measure. Micronaire remains the industry standard for indicating fineness and maturity because it is the easiest and fastest measurement available.

Micronaire has a defined effect of cotton pricing (Table 4.1.2). The micronaire price adjustments established for the 2013 cotton loan rates demonstrates that low micronaire can be penalized in price much more than high micronaire values. The USDA classing office in Rayville, LA reported that more than half of the cotton passing through Rayville's classing office had a micronaire to which a discount on quality can be assessed (Quality of cotton classed by the classing office, 2014).

Table 4.1.1. Classification levels of fiber fineness and maturity ratio

Fineness (mtex)	Class	Maturity ratio	Class
<135	Very Fine	<0.7	Very Immature
135-175	Fine	0.7-0.8	Immature
175-200	Average	0.8-1.0	Mature
200-230	Coarse	>1.0	Very Mature
>230	Very Coarse		

Table 4.1.2. Classification of micronaire and corresponding price adjustments

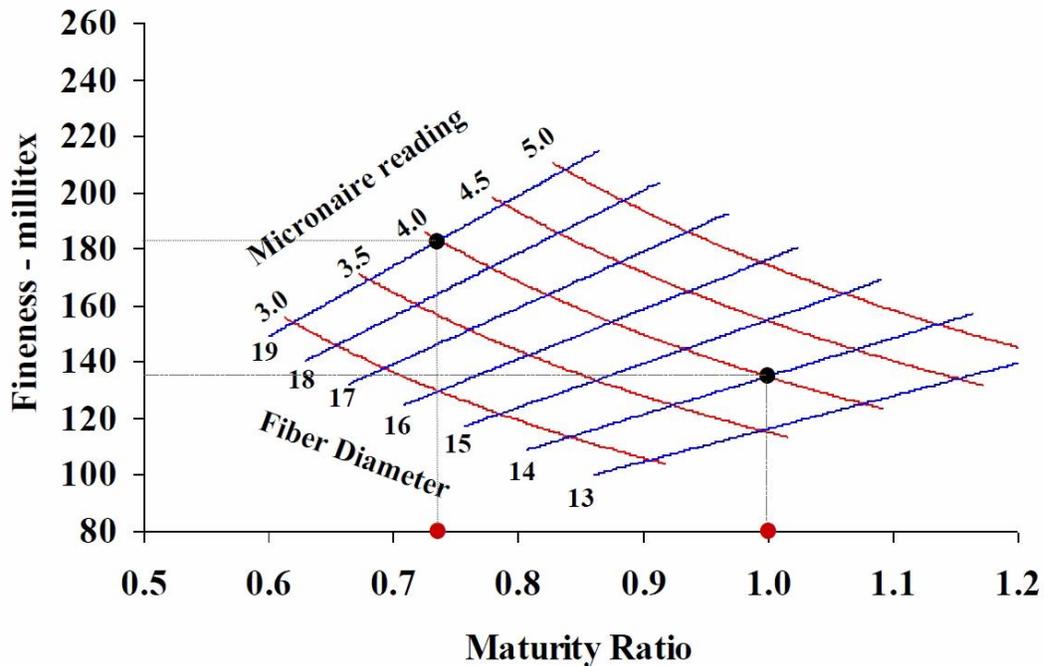| Micronaire	Class	Points pound^{-1} adjustment (2013)	Points pound^{-1} adjustment (2014)
<3.4	Low Discount	-950 (<2.4) -900 (2.5-2.6) -605 (2.7-2.9) -340 (3.0-3.2) -170 (3.3-3.4)	-950 -900 -600 -340 -170
3.5-3.6	Low Base	0	0
3.7-4.2	Premium	+15	+15
4.2-4.9	High Base	0	0
>5.0	High Discount	-270 (5.0-5.2) -385 (>5.3)	-285 -410

Figure 4.1.1. Relationship between micronaire and fiber fineness (mtex) and maturity ratio according to the Lord equation (Lord, 1956) *Image courtesy of E. Hequet (Texas Tech University, Lubbock, TX)*

The intent of this study is to examine within genotype variability for the traits: micronaire, maturity ratio, and fineness. The maturity ratio of cotton fibers is determined by the degree of secondary cell wall development; a process whose rate is largely dependent on accumulated heat units during the window for fiber development (Gipson, 1986). Fiber maturity influences the dye retention of fibers for textiles. Immature fibers have thinner cell walls and do not hold dyes to the same degree as fully mature, thick walled fibers. When immature fibers are present in a large fraction the resulting textile is subject to inconsistent dying. Dying imperfections can result in the yarn not meeting a buyer's contract specifications and a major loss for a textile processor.

Fiber fineness and maturity can be measured directly through several methods each with specific advantages and disadvantages. The current Standard Test Method for measuring fiber maturity is via Sodium Hydroxide swelling and subsequent quantification via polarized light microscopy (ASTM 1442-06, 2000). The caveats to this method are that the process is subjective in quantifying mature vs. immature fibers and also the act of swelling fibers alters the natural conformation in which fibers are processed. The test requires skilled technicians and is time-consuming.

38

Fiber fineness, after fiber strength and length, is one of the most important fiber quality traits in predicting yarn strength in rotor or ring spinning systems (Jackowski et al., 2002). This trait is the primary factor in how many fibers can fit into a given width of yarn and knowing this value can allow for prediction of yarn yield. Finer fibers increase inter-fiber surface contact, increasing cohesion which improves yarn strength (Morton, 1993). Fineness is a major determinant for the degree of twist required to achieve a specific yarn quality. Despite the direct importance of fineness, industry and cotton scientists most often use MIC to indicate fiber fineness simply ignoring the confounding interaction from the samples' maturity ratios.

Variation in fiber quality by boll position on a plant can be determined using box or position mapping. In box mapping, individual fruiting positions are harvested by hand and categorized based upon their nodal position and their position along the fruiting branch. The technique has been used in many studies to examine fiber development and test treatment effects on the distribution of individual cotton bolls, yield, and quality (Jenkins et al, 1990a; Mauney, 1984; Bradow et al., 2000; Hague et al., 2014).

Meredith and Bridge described in 1973 a method of harvesting which has a similar effect of partitioning bolls based upon the time they opened. In their research, open bolls were harvested from plots at one-week intervals for analysis. Sampling in this way bulks positions into zones based on their maturation and opening window similar to zone mapping at the end-of-season (Hague et al., 2014) but differs in that interval harvesting reduces the weathering of early bolls. Both studies found that fiber quality changed across the crop's architecture. Hague et al. (2014) was unable to find a consistent trend between bottom/middle/top fruiting zone and micronaire. It is suspect that node*position interaction prevented detection. Meredith and Bridge described that micronaire decreased with the last two harvest events (Meredith and Bridge, 1973).

The Cottonscope is a new instrument designed to optically measure the parameters of fiber fineness and maturity of cotton fibers rapidly and inexpensively (Rodgers et al., 2012a). Samples are

prepared by chopping fiber samples into 'snippets', and 50±0.2mg is weighed then suspended in an ionized water bath. The water bath is agitated to allow individual snippets to pass across a microscope lens illuminated with a polarized light source. Images are captured and analyzed by the instrument until 20,000 individual fiber snippets per sample are measured. The Cottonscope is considered to be more precise than HVI as well as less expensive and faster than AFIS. The Cottonscope was designed from the ASTM procedure of quantifying cotton Maturity Ratio via polarized light (Rodgers et al., 2012a; Paudel et al., 2013).

The Cottonscope was developed by CSIRO by combining the technology of the CottonScan™ to measure fiber fineness and the SiroMat™ to measure maturity in an automated system. The software calculates fiber fineness by measuring the length of 20,000 snippets and dividing by the weight of the sample. This process is more precise than the hand-grading method first described by Pierce and Lord (1928) in which a sample was cut into cm long snippets, counted, and weighed to find the "Hair Weight" (average weight per cm of fibers). The Cottonscope's advantage in finding fineness is in its ability to rapidly accurately measure the precise length of thousands of fibers snippets and calculate this with a weight measured to great precision. The speed with which the instrument operates allows for many replications in measuring a cotton sample during the time it would take for a single hand-grading to be performed.

Fiber maturity calculations are simultaneously calculated using the Cottonscope. Birefringence is a property of crystals to change the direction of polarized light as the light passes through the crystal. This relates to cotton fibers because they are >90% crystalline cellulose (Haigler, 2009). The Cottonscope optically measures the cell's external (cell surface) and internal (lumen) perimeter using birefringence and polarized light microscopy (ASTM D1442, 2000). The refractory index of crystalline cellulose makes it possible to measure the cell wall thickness based on changes in direction of polarized light refracting as it enters the cellulosic cell wall and again as it enters the lumen (Wolman, 1975). The intensity of birefringence clearly differentiates the hollow space of the lumen and the dense cellulosic cell wall. For

each of the 20,000 fiber snippets, the degree of thickening (Ө) is calculated to determine the ratio of mature and immature fibers. A histogram is displayed, and the maturity ratio for the sample is recorded.

The number of bolls per plant, relative position of bolls, boll weight, and total weight within a genotype all impact the source/sink dynamic. Examining both the distribution of yield and fiber quality together will provide insight into how the two properties of a crop are related. Productivity and profitability are both dependent on yield and quality, thus understanding the relationship between them all is important to breeding efforts and agronomic practices.

4.2 Materials and Methods

In 2013 and 2014, five cotton genotypes were grown in the field in two-row plots in both Alexandria and St Joseph, LA. The experimental design was a randomized complete block with four replications. Plant spacing was 0.97m (38 inches) between rows with a within row spacing of 0.1m (3 plants/ft.). The crop was managed according to LSU AgCenter Best Management Practices at each site with regard to fertility, weed and insect control for a conventional (non-transgenic) cotton crop. Three genotypes represented unreleased, elite genotypes developed by the Louisiana State University cotton breeding program, and two were commercially developed conventional cotton varieties. Plants were chemically defoliated at approximately 80% open bolls. Two weeks after defoliation, five plants were randomly selected within each plot in order to map boll distribution. Plants were clipped at the soil surface and the cotyledonary node (0) used as a reference point. Bolls were hand-harvested and combined across the five plants within replication according to their fruiting position (Jenkins, 1990a and 1990b, and Conkerton et al., 1993). Boll count by fruiting site was recorded as plants were harvested. This process was for each genotype.

Table 4.2.1 Five genotypes examined for within-plant fiber quality variation in Alexandria, LA and St. Joseph, LA in 2013 and 2014

Louisiana adapted lines	Control lines
LA10307140	DP393
LA10307108	FM958
LA10307021	

The five plants were harvested and seed cotton from a fruiting site (node by position) was bulked from all plants within plots (Jenkins et al., 1990a and 1990b, and Conkerton et al., 1993). Ginning was performed using a 10-saw laboratory gin (Dennis Manufacturing Co, Athens, TX) to separate the seed from fiber at each fruiting site in a plot. This also served to homogenize the fibers from across plants and within bolls before the sample underwent quality testing.

Samples were equilibrated to standard environmental conditions. For forty-eight (48) hours, fiber samples were held at a temperature of 70($\pm$)1°F and relative humidity of 65($\pm$)2% (ASTM D1776/D1776M, 2015). Because of the hygroscopic nature of cotton fibers, fineness is affected significantly by conditioning fiber samples at different temperatures and RH (Rodgers et al., 2012a). After conditioning, samples were measured using the Cottonscope, and a partial set of samples (those that could meet the 3.24g weight requirement) were measured using a Fibronaire (Motion Control, Inc., Dallas, TX; ASTM D1448, 1997). Each fruiting site was subsampled three (3) times, and the Cottonscope measured two (2) replicates for each subsample. The Cottonscope gives values of maturity ratio (ratio of mature and immature fibers within a sample) and fineness (mtex). Samples with sufficient weight (3.24g) had micronaire assessed with three (3) replicates using the Fibronaire. Fibronaire is the cotton industry standard method to measure micronaire by airflow resistance upon which HVI micronaire is based.

Both instruments were calibrated using reference cottons and validated each day before use. The Fibronaire arrives at the value for Micronaire by air flow resistance which is not a direct physical

measurement. Therefore, calibration cottons were tested to be sure that the Micronaire readings were within proper range. The Cottonscope optically measures the physical fiber properties within a sample, but validation of the accuracy and precision is achieved by testing calibration cottons well-defined via image analysis for fineness and maturity ratio. A set of three, highly-uniform calibration cottons were used to check the two instruments performance each day before experimental samples were measured. Calibrations using well-defined and unbiased reference materials for the fiber quality parameters are needed to maintain the accuracy and precision of data from testing instruments (Hequet et al., 2006). The average values from subsampling and replicated runs of the Cottonscope were used to show the trends associated with changes in fiber quality plotted against fruiting position. Statistical analysis similar to a previous box mapping study was performed (Conkerton et al., 1993). Analysis of Variance (ANOVA) was calculated via the Mixed Procedure in SAS 9.4 in order to partition the variance associated with each factor. The Univariate Procedure was used to plot frequency distributions of fiber quality within each genotype.

4.3 Results

Analysis of the genotype, node, and position effect on fiber fineness, maturity ratio, and MIC used the MIXED model procedure in SAS 9.4. The experimental design was a repeated measure in space. Each of the three fiber quality values were significantly affected by the genotype, node, and position from which they were taken. Interactions of genotype*node and node*position were significant for each of the measurements. No significant interaction of genotype*position or the three-way interaction of genotype*node*position was detected (Table 4.3.1). The effect of year and location was not significant for the quality traits examined. Based on this, histograms were generated across years and locations.

Categorical differences between genotypes in fiber fineness were observed (Fig 4.3.1). As with MR, genotypes DP393 and LA10307021 stand out as extremes. DP393 has the coarsest mean fiber fineness (210.18) and LA10307021 has the finest mean (178.36). LA10307021 is ideal for spinning systems which require finer fiber inputs. When examining the within plant distributions, this still holds

true. LA10307021 has the lowest kurtosis (1.20) whereas DP393 has the highest (3.308). DP393 and

LA10307021 both have a negative skew however LA10307021 has the highest skewness (-0.463) and

DP393 one of the lowest (-1.125). This tells us that DP393 is a coarser type fiber with a flatter

distribution skewing more heavily toward finer fibers. When examining the mean, the sample may appear

adequately fine, however with these distribution statistics we know that the mean is weighted by a larger

negative skew and the fibers are less tightly distributed around the mean. For spinning systems requiring

not just fine fibers but uniformly finer fibers, LA10307021 would be ideal. Without distribution statistics,

these observations are not possible. Mean value traits are helpful in bale selection, but within-sample

variation can influence spinning consistency even when mean values do not differ.

Table 4.3.1 Analysis of variance for fineness (mtex), maturity ratio, and Fibronaire micronaire from five genotypes grown in Alexandria, LA and St. Joseph, LA in 2013 and 2014 sampled by fruiting site (node by position) of bolls

		Fineness	Maturity Ratio	Fibronaire	
Source	DF	MS	MS	DF	MS
Genotype	4	8695.77**	0.078**	4	4.57**
Node	20	8363.01**	0.123**	15	0.98**
Genotype*Node	66	743.06**	0.011**	53	0.33**
Position	2	26315**	0.227**	2	6.81**
Genotype*Position	8	356.04	0.005	8	0.23
Node*Position	28	1076.45**	0.019**	19	0.36**
Genotype*Node*Position	86	387.52	0.005	55	0.21
Year	1	722.59	1.250	1	6.35
Location	1	56578	0.033	1	19.08
Year*Location	1	672.22	0.158**	1	2.57*
rep(Year*Location*Genotype)	32	1436.69**	0.018**	32	0.46**
Residual	867	359.68	0.004	404	0.15

*Statistically significant at p<0.05.
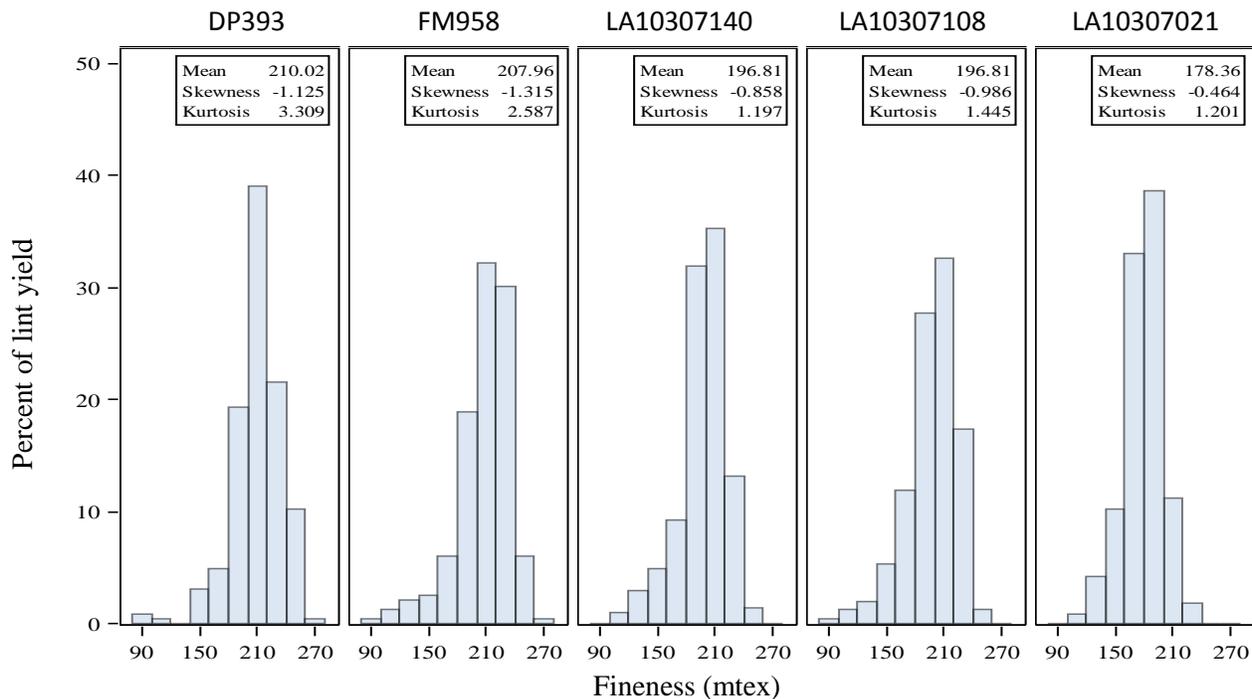**Statistically significant at p<0.01

Figure 4.3.1. Distribution of fiber fineness (mtex) within genotypes grown in Alexandria, LA and St. Joseph, LA (2013 and 2014)

When examining fiber MR by genotype there are slight differences between the mean values for each genotype (Figure 4.3.2). More notable is the differences in the skewness and kurtosis between genotypes. LA10307021 had the lowest kurtosis (3.944) and highest skewness (-1.533) values. Theoretically, this tighter distribution about the mean and less negative skew in maturity would translate to a more consistent spinning and dying. Conversely, DP393 has the highest kurtosis (19.995) and lowest skewness (-3.472) values.

LA10307021 had the most ideal mean micronaire and within-plot distribution. With the lowest mean (4.27), lowest kurtosis (0.434), and one of the highest skewness (-0.665) (Figure 4.3.3), LA10307021 appears to have not only the most ideal mean but also a tight distribution about the mean. DP393 and FM958 quality distributions are also very telling of their consistency. Both have a high mean MIC (4.934 and 4.941 respectively), however DP393 has the highest skew (-0.47) and lowest kurtosis (0.435) of all genotypes examined. FM958 has the largest negative skew (-1.186) and largest kurtosis.
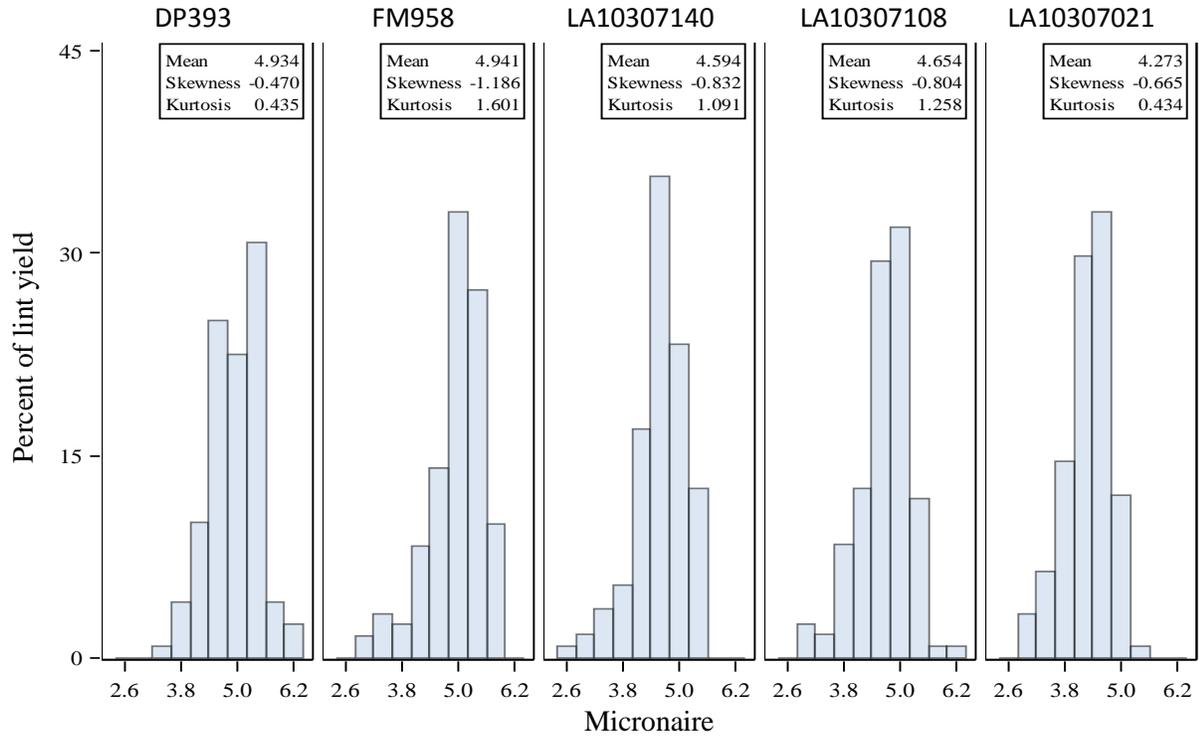
45

Figure 4.3.2. Distribution of fiber micronaire within genotypes grown in Alexandria, LA and St. Joseph, LA (2013 and 2014)
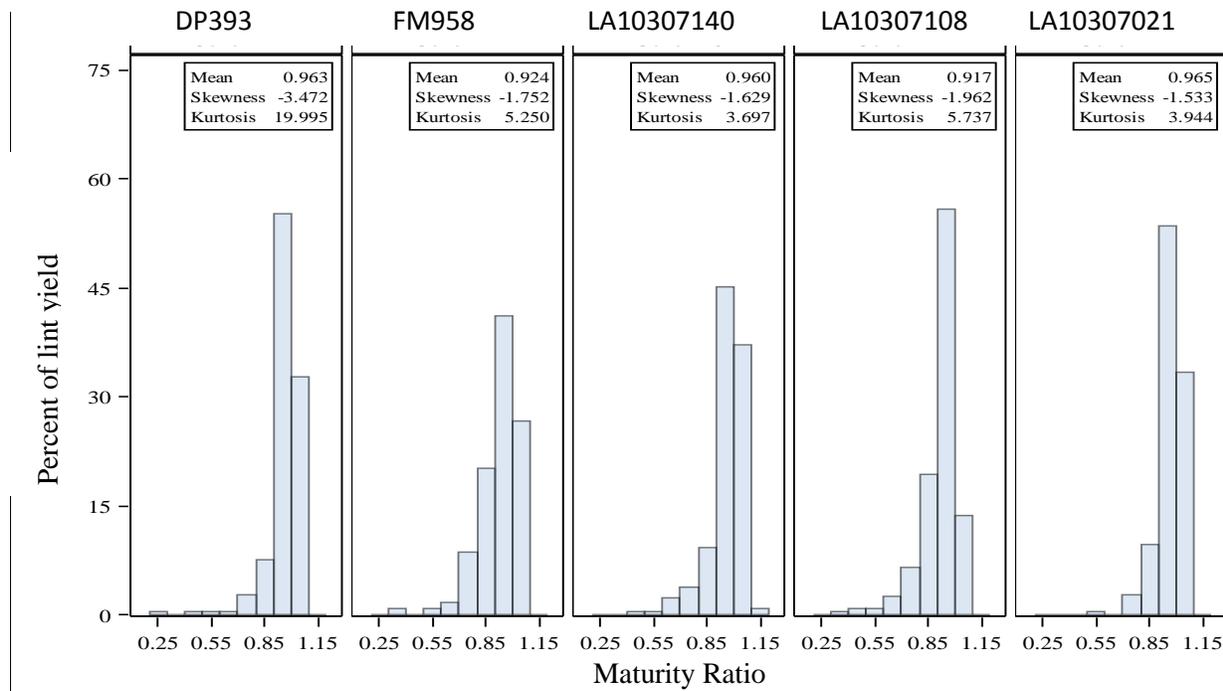

Figure 4.3.3. Distribution of fiber maturity ratio within genotypes grown in Alexandria, LA and St. Joseph, LA (2013 and 2014)

46

Reference lines on figures 4.3.4 and 4.3.5 indicate the classification ranges for each fiber trait.

Figure 4.3.4 illustrates that fineness decreases by horizontal position (averaged across vertical nodes) is a

trend present within all genotypes. The average fineness was lower as bolls extended outward on the
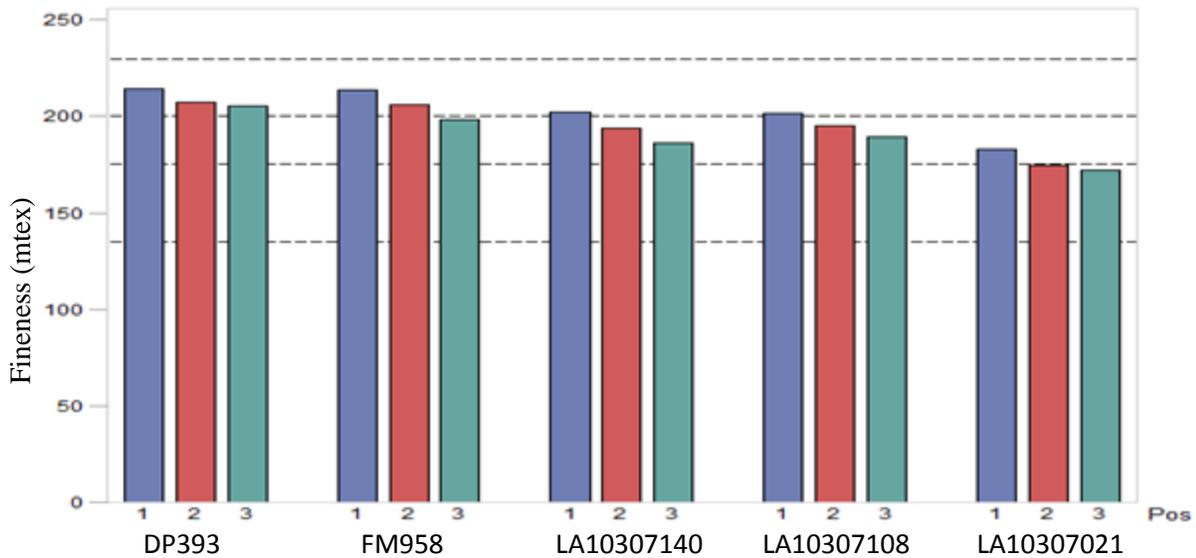
sympodial branches.

Figure 4.3.4. Fineness (mtex) by horizontal position within genotype grown in Alexandria, LA and St. Joseph, LA (2013 and 2014)
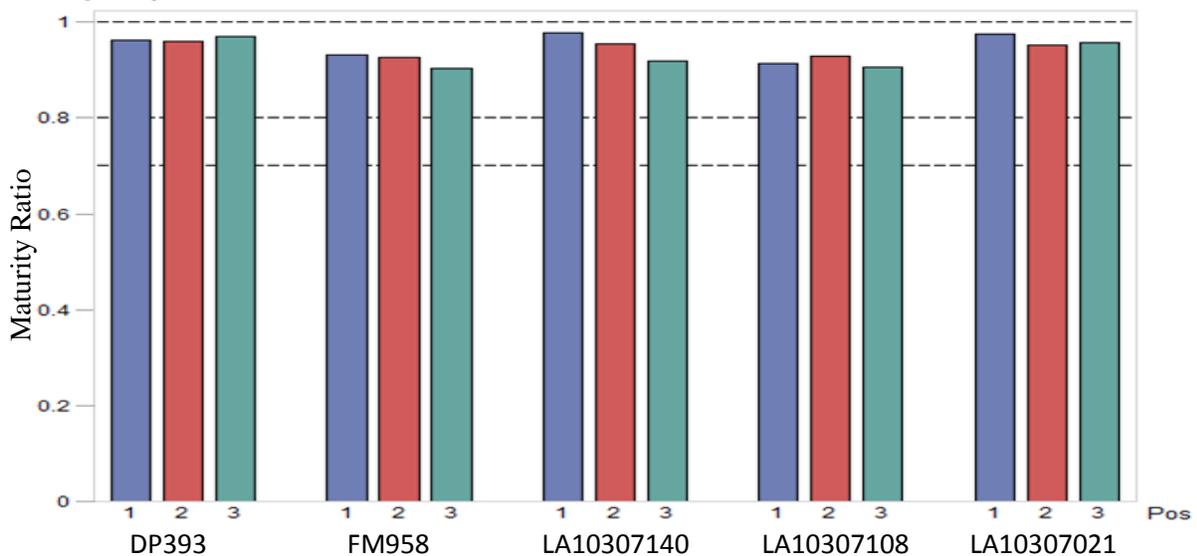

Figure 4.3.5. Maturity ratio by horizontal position within genotype grown in Alexandria, LA and St. Joseph, LA (2013 and 2014)

ANOVA showed that position was a significant source of variation in maturity ratio. Unlike fiber fineness, a linear trend in horizontal positions across all genotypes was not present in MR. Figure 4.3.5 shows that MR was not linearly affected by the horizontal position on a branch.

Table 4.3.2 expresses the variation for each fiber quality attributed to each source as a percent of the total mean square. Examining variation within each trait reveals the degree to which each trait is affected by each source. One goal of this research was to determine the value of the Cottonscope for fiber quality improvement. Due to both the large environmental influence on micronaire and the confounding interaction of fiber fineness and fiber maturity, it was possible that the traits would independently allow for selection gains greater than that of micronaire. The results in table 4.3.2 show that micronaire has the highest percentage of variation due to genetic factors (10.86%) whereas genetic variance in fineness (8.23%) and maturity ratio (4.08%) were less. The environmental effects present a problem with selection gains in these traits. Micronaire has a large year effect (15.08%) whereas fineness is very low (0.68%) The location effect is the largest source of variation for fineness (53.52%) and MIC (45.32%), but this could be accommodated by making selections within a single location. Maturity ratio shows several problems for breeding selection. The genetic effect on MR is 4.08% meaning selection for this trait would be slow relative to the other traits. In addition, the year (65.34%) and year*location (8.26%) further complicate any potential progress.

Table 4.3.2. Variation as a percent of total mean square of fiber fineness, maturity ratio, and micronaire within five genotypes grown in Alexandria, LA and St. Joseph, LA (2013 and 2014)

Source	Fineness	Maturity Ratio	Micronaire
		% of Total MS	
Genotype	8.23	4.08	10.86
Node	7.91	6.43	2.33
Genotype*Node	0.70	0.57	0.78
Position	24.89	11.87	16.18
Genotype*Position	0.34	0.26	0.55
Node*Position	1.02	0.99	0.86
Genotype*Node*Position	0.37	0.26	0.50
Year	0.68	65.34	15.08
Location	53.52	1.72	45.32
Year*Location	0.64	8.26	6.10

Table 4.3.3. Correlation analysis of within genotype fiber quality and yield components within five genotypes grown in Alexandria, LA and St. Joseph, LA (2013 and 2014)

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1 Bolls per plant	1.00							
2 Boll weight (g)	-0.59*	1.00						
3 Lint weight (g) per plant	0.76**	0.06	1.00					
4 Lint percent	0.15	-0.02	0.15	1.00				
5 Lint weight (g) per plot	0.76**	-0.06	0.87**	0.49	1.00			
6 Maturity ratio	-0.66*	0.70**	-0.25	-0.09	-0.29	1.00		
7 Fineness	-0.03	0.02	-0.01	-0.34	-0.17	0.03	1.00	
8 Micronaire	-0.15	-0.01	-0.19	-0.37	-0.28	0.18	0.88**	1.00

*Statistically significant at $p<0.05$.
**Statistically significant at $p<0.01$

Significant correlations with yield, yield components, and fiber maturity ratio were detected (Table 4.3.3). Fiber MR was negatively correlated with bolls per plant and positively correlated with boll weight. Samples with lower maturity ratio came from lower weight bolls and plants with more bolls. The correlation of micronaire and fineness was significant and very high (r-0.88). The correlation of micronaire and MR was not significant and low (r=0.18).

4.4 Discussion

This study found both node and position to be a significant source of variation for these two quality traits. The Cottonscope's ability to measure single bolls at each node and position detected a significant interaction between genotype*node and node*position. These interactions indicate that node effect on fiber fineness and maturity ratio differs by the genotype examined. The interaction of node*position means that fruiting site within a plant significantly influences fineness and maturity ratio. Proper sampling method will be required to maximize the ability to discern genetic differences between samples of segregating populations. The results confirm those reported in previous studies that attribute boll position as a factor influencing fiber quality (Meredith and Bridge, 1973; Zhao and Oosterhuis, 2000; Bauer et al., 2009; Hague et al, 2014). Bauer et al. (2009) found significant differences between fineness and maturity ratio of first position bolls averaged over all vertical nodes within the two genotypes tested via AFIS.

The study by Zhao et al. (2000) was unable to examine quality distributions of its experimental samples or look into individual boll positions because of the required sample size for HVI. Significant differences were reported between first and second position bolls combined across vertical zones (upper, middle, low zones). The major focus in the work was the statistical differences in values for fiber strength. Data was presented showing significant effects for fiber MIC resulting from boll position however it was not addressed further (Zhao et al., 2000). A study of cotton fruiting zones effect on fiber quality did not find zones to be significantly different (Hague et al., 2014).

In this study, there was no significant correlation between fineness and maturity ratio (r=0.18) (Table 4.3.3). Ulloa (2006) found a significant correlation in two populations of F2:3 and F2:6 progeny (r=0.86 and 0.80 respectively) derived from a cross of FiberMax 832 and MD51ne. AFIS was used to examine fineness and maturity ratio in the study. While a significant bias has been reported in AFIS measurements (Montalvo, 2007), this is not likely to account for such a large difference between this study and Ulloa (2006). More influential is the difference in populations and experimental designs.

Louisiana adapted lines had the more ideal fiber qualities than the commercial checks in this research. All genotypes tested had a mean MR at the categorically mature level (0.8-1.0). Mean values may not provide enough information about a fiber sample to be an ideal predictor of spinning performance. Currently, maturity ratio is divided into crude classifications but the tolerances of dye retention and spinning performance at each level is not well defined. The differences seen in the distributions are where problems may be identified. DP393 is most likely to have inconsistent dying due to its highest immature fiber content. While the mean MR of LA10307021 (0.965) and DP393 (0.963) are very similar, their distributions are very different. It is the consistency of the raw fiber input that factor into spinning consistency. Information on the tolerance level of immature fiber content would benefit breeders. With this information, intense selection for low MIC to develop finer fibers can be performed because the indirect effect of lowering maturity may not have a significant effect on spinning performance.

Comparing the fineness and MR distributions with the MIC distributions, we see that MIC fails to provide a full indication of the spinning value even when examined as a distribution. The interactions of fineness and MR within DP393 confounded its MIC values making it appear less variable than it actually is. Fibers from DP393 do have the highest mean MIC, but the value is below the discount range thus they are still desirable for spinning. Even the MIC distribution appears good. It is only when we examine fineness and MR independent of MIC that the variation within this genotype is so large. The variation is a likely indicator that consistency in spinning would not be ideal.

Based on the percent variation for each trait, selection for fineness would be more effective than selection for maturity ratio. While the location effect for fineness was high, the year and year*location effect was minimal. The implication here is that fineness would be ideal for selection within the same environment year to year. Maturity ratio and micronaire all had a large year and year*location effect, thus the effect of selection would be limited due to environmental interactions year to year.

The separation of micronaire into fineness and maturity has the advantage for genetic selection because selection for lower fineness genotypes could be made and the impact on maturity could be monitored. Selecting lower micronaire to improve fineness would inadvertently lower maturity ratio. While lower fineness values are preferred for improving yarn spinning, lower maturity negatively impacts yarn quality.

Breeding is not able to focus solely on single traits. Inter-trait linkages have the consequence that as one trait is modified, unintended and often negative changes in other traits can occur. Desalegn (2009) reported a negative correlation of fiber fineness with boll weight (lower fineness correlates with higher boll weight), and a positive correlation of fineness with bolls per plant, lint yield, and lint % (lower fineness correlates with less bolls per plant, lint yield, and lint %). In that study, fiber fineness is described, however micronaire was the only measure taken to indicate this. Therefore, it is impossible to determine if fineness alone and not underlying maturity in the samples were involved in these correlations.

A negative relationship between maturity ratio and bolls plant^{-1} and positive relationship of maturity ratio to average boll weight (Table 4.3.3) but no relationships were significant between fineness or micronaire with any of the yield components. It is logical that if the number of bolls available as sinks decreases then the maturity of the fibers remaining would have more resources at to utilize. The result is an increase in fiber maturity. Also, the very strong positive relationship between average boll weight and fiber maturity suggests that fiber maturity is a significant factor in determination of boll weight.

This information supports the need for breeding studies to determine the success of improving micronaire, fineness, and maturity ratio distributions on cultivars and the unit change effect on lint yield. The interaction between genotype and node indicate a potential for bias in sampling different genotypes. Internode distances vary between cultivars thus the same Pos(Node) is situated at different levels for different cultivars. Considerations in sampling methods are needed to ensure that genotypes with different fiber quality values are detected. Meredith and Bridge (1973) found plot sampling protocol can introduce significant bias into genotype evaluation. Sampling should be consistent between genotypes and representative of the total bolls within a plot.

Modern fiber testing equipment which provides fiber quality distributions would be useful to determine tolerances by spinning systems. Breeding goals aim for longer, stronger, and finer fibers but without a strong understanding of the tolerances spinning systems these goals are likely unbalanced in their focus. Examining how the variation within genotypes affects the spinning performance would provide a more concrete objective for breeding programs to develop high-quality lines. The Cottonscope shows certain useful advantages over traditional micronaire measurements. As cotton breeders and geneticists examine and introduce exotic germplasm for sources of unique traits, the Cottonscope presents major advantages. Micronaire measurements are limited in range from 2.2-8.0 and are calibrated within an even smaller range. Cotton species with extremely fine or coarse fibers easily fall outside of these ranges.

The results of this study are unprecedented; fiber fineness and maturity ratio have not been examined by individual fruiting positions in earlier literature. Previous studies seeking to examine within-plant variation of fiber quality have been limited in several ways. Instruments readily available prior to the Cottonscope have been too expensive, imprecise, require too large a sample size, or were significantly biased in their measurement to examine within-plant variation with this degree of resolution. The findings in this work showed: 1) that selection for micronaire is adequate for reducing fiber fineness in environments where fiber maturity is not a problem. 2) The variation present in fiber fineness and maturity ratio due to a boll's node and position require consistent boll sampling to maximize selection efficiency for these traits. 3) The importance of micronaire, fineness, and maturity ratio as well as the uniformity within-bales remains poorly understood regarding its impact on spinning performance.

The Cottonscope was shown to be a rapid and precise tool for better understanding micronaire and its components of fineness and maturity. This will be a valuable tool in bridging breeding efforts for improved quality and demands of the textile industry; however micronaire still remains the fastest, least expensive way for cotton breeders to evaluate the thousands of samples generated each year.

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Vita

Matthew Oliver Indest, a native of Southeast Louisiana, is an avid outdoorsman and homesteader who took interest in gardening as a hobby during high school. His interest evolved to a foray into the business of landscape installation and maintenance as well as a student worker with the sweet potato breeding program in order to support his family with the birth of his son, Marcus, in 2010. In 2011, Matthew earned his bachelor's degree in Horticultural Science from Louisiana State University. Upon graduation, his interest in agricultural systems management and plant breeding led him to take an assistantship with the cotton breeding program and spend 18 months as the resident horticulturalist with the LSU AgCenter Botanic Gardens and Museum at Burden Plantation. During this time his graduate work has been in pursuit of a doctorate in plant sciences and he anticipates completion in 2015. After graduating, Matthew plans to stay within Southeast Louisiana to continue working in the applied agricultural sciences.