

## QUALITY MEASUREMENTS

### Small-Sample Cotton Fiber Quality Quantitation

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#### INTERPRETIVE SUMMARY

The price of cotton fiber and the monetary return to the cotton grower depend on fiber yield and quality, both of which are set by crop management practices in interaction with the growth environment. Fiber yield is easily quantified in bales per acre, but fiber quality is a complex of both qualitative and quantitative properties like fiber length, length uniformity, fineness, maturity [measured as micronaire], strength, color, and trash content. Measurement of fiber quality is further complicated by significant natural and environment-related variations in fiber shape and maturity at the bale, plant, boll, and seed level. Thus, improvements in fiber quality will best be achieved through optimization of the bulk fiber properties determined during cotton classing and through increasing fiber quality uniformity.

The U.S. textile industry has proposed, defined, and quantified several premium and discount price ranges for bulk fiber qualities. A predictive model of fiber processing potential (Engineered Fiber Selection Cotton Fiber Management System plus GINNet)<sup>1</sup> is being developed, using bale-level length and micronaire values provided in USDA fiber-classing high volume instrument data. Textile processors and mill buyers are setting stricter fiber quality requirements, and successful cotton

producers are looking beyond yield enhancement to modified production, harvest, and ginning practices that will allow them to meet increasing demands for cotton fiber with specific qualities. However, cotton fiber quality measurements at the boll, locule, or seed level are limited by the large sample-size requirements of commercial cotton fiber testing instrumentation and inherent biases and high costs in time and labor of non-instrumental measurement methods.

In the research described here, a specialized airflow particle-sizer (AFIS) was used for rapid measurements of the characteristics of small fiber samples (500–10 000 fibers per sample) from Upland or Pima bolls of chronological maturities ranging from 21 d after bloom date to natural boll opening at 56 d after flowering in Starkville, MS. Fiber-quality properties were mapped according to open-boll position for one Upland cotton variety, Pee Dee 3, grown in Florence, SC. Each AFIS sample analysis, which requires less than 5 min, produces a 19-factor data set that includes sample means of length, diameter, area, circularity, and the associated distribution percentages, that is, short fiber contents from fiber length measurements and immature fiber fraction and fine fiber fraction from distributions of circularities and cross-sectional areas, respectively.

This report, which is based on data subsets from field studies of Upland and Pima cottons grown in South Carolina and Mississippi in 1992, and 1993, describes the use and potential of AFIS in generating replicated fiber-quality data and maps

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<sup>1</sup> Trade names are necessary to report factually on available data. The USDA neither guarantees nor warrants the standard of the product or service, and the use of the name USDA implies no approval of the product or service to the exclusion of others that may be suitable.

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**Abbreviations:** HVI, high volume instrument; AFIS, Advanced Fiber Information System incorporating the AFIS length and diameter module and the AFIS fineness and maturity module;  $\mu$ AFIS, micronAFIS, AFIS-F&M micronaire analog;  $A(n)$ , cross-sectional area by number; Ca-XRF, calcium weight ratio quantitation by x-ray fluorescence;  $D(n)$ , diameter by number; dpa, days post [flower] anthesis; FFF, fine fiber fraction; IFF, immature fiber fraction;  $L(n)$ , length by number;  $L(w)$ , length by weight;  $SFC(n)$ , short fiber content by number;  $Sfc(w)$ , short fiber content by weight;  $\theta$ , fiber circularity, *Theta*.

appropriate for studies of the complex relationships among growth environment, production practices, and fiber quality. Significant variations in Upland cotton fiber lengths, diameters, cross-sections, fiber-filling, and maturity were detected at the boll and locule levels. The particle sizer, with a theoretical sample size of 1 to 10 000 fibers, is a powerful new, quantitative tool that is currently being used in the development of predictive cotton fiber quality models and component analyses of the bulk fiber qualities that determines marketability and utility value of a cotton crop.

### ABSTRACT

**Cotton [*Gossypium* spp.] fiber quality quantitations at the boll, locule, or seed level are limited by the large sample-size requirements of commercial cotton fiber testing instrumentation and inherent biases and high costs in time and labor of non-instrumental measurement methods. Quantitative examinations of the natural and environmentally induced variations in fiber properties were performed at the boll or locule level or during fiber development by analyzing small samples [ $\geq 500$  fibers per sample] with a specialized airflow electro-optical particle sizer capable of rapid measurements of fiber lengths and physical maturities. The fiber samples examined were from Upland [*G. hirsutum*] or Pima [*G. barbadense*] bolls of chronological maturities ranging from 21 d post anthesis to natural boll opening. Significant variations in Upland cotton fiber lengths, diameters, cross-sections, circularities, and maturities were detected at the boll and locule levels, and these fiber-quality parameters were mapped according to open-boll position for one Upland cotton genotype. The particle sizer, with a theoretical sample size of 1 to 10 000 fibers, is a powerful new, quantitative tool for use in the development of predictive cotton fiber quality models and component and variability analyses of the bulk fiber qualities that determines marketability and utility value of a cotton crop.**

The monetary return to the cotton producer depends on fiber yield and quality, both of which are determined by crop management practices and growth environment (USDA, 1980; Munro, 1987). Fiber yield is quantified in kg ha<sup>-1</sup> (or customarily bales per acre), but fiber quality is a composite of both qualitative and quantitative parameters, for example, fiber length, length

uniformity, fineness, maturity, (as micronaire), strength, color, and trash content (ASTM, 1988). Quantitation of fiber quality is further complicated by significant natural and environment-related variations in fiber shape and maturity at the bale, plant, boll, and seed level (DeLanghe, 1986; Bradow et al., 1994; Davidonis and Hinojosa, 1994). Thus, improvements in fiber quality will best be achieved through optimizing the bulk fiber qualities determined during cotton classing and through increasing fiber quality uniformity.

In the U.S. textile industry, several quantified premium and discount price ranges for bulk fiber qualities have been proposed (Deussen, 1992; Deussen and Faerber, 1995). A predictive model of fiber processing potential (Engineered Fiber Selection Cotton Fiber Management System plus GINNet) is being developed, using bale-level length and micronaire values provided in USDA fiber-classing high volume instrument (HVI) data (Chewning, 1994). Textile processors and mill buyers are setting stricter fiber quality requirements, and successful cotton producers are looking beyond yield enhancement to modified production, harvest, and ginning practices that will allow them to meet increasing demands for cotton fiber with specific qualities.

However, the complex relationships among fiber quality characteristics, natural fiber variability, and growth environment, including weather and production practices, are normally described at the bale level in terms of bulk or composite fiber qualities that may not adequately represent or quantify the magnitude and distribution of the significant variations in quality that are characteristic of a biological fiber like cotton. New strategies for improving fiber quality and increasing fiber-quality uniformity within premium price ranges require rapid, reproducible, replicated fiber-quality quantitation of small samples (i.e., the fibers from a single boll, locule, or seed). Replicated fiber-quality quantitations are also necessary for the development of predictive models comparable to the whole plant growth and yield simulations used by producers (Baker et al., 1983; Lemmon, 1986), and for bridging the current gap between cotton harvest-value (Sequiera, et al., 1994) and Engineered Fiber Selection-processing simulations (Chewning, 1994).

Currently, instrumental fiber-quality quantitation methods, for example, HVI used in U.S. cotton classing, require blended, randomly collected fiber samples of sizes (>3 g) that significantly exceed single-boll fiber weights (USDA, 1980; ASTM, 1988). Randomly selected, composite samples of approximately 100 g are recommended for acceptance and laboratory fiber testing, whatever method is used (ASTM, 1989a). Commercial fiber-testing instruments provide estimates of large-sample composite averages of important fiber qualities such as fiber length and micronaire (Ramey, 1982; Munro, 1987; Lord and Heap, 1988; Deussen, 1992; Behery, 1993; Deussen and Faerber, 1995). Alternative non-instrumental methods, particularly single-fiber and microscopic analyses, are extremely time-consuming and subjective, as well as being subject to serious bias when cost constraints limit replication or when truly representative subsamples are not randomly selected (Munro, 1987; ASTM, 1989b).

The fiber qualities, length, fineness, and circularity (degree of secondary cell wall thickening), are shape and size quantities. Thus, these fiber qualities are amenable to instrumental measurement by electron-optical particle-sizing. The AFIS equipped with length and diameter module measures the lengths and diameters of individualized fibers (Behery, 1993; Bragg and Shofner, 1993; Bradow et al., 1994). The prototypic AFIS fineness and maturity module quantifies fiber cross-sectional areas and circularities. Fiber perimeter can be calculated by AFIS fineness and maturity module from the cross-sectional area and circularity, and another AFIS fineness and maturity algorithm is used to calculate micronAFIS ( $\mu$ AFIS), which is closely analogous to micronaire as measured by HVI in U.S. cotton classing (USDA, 1980). An optimized minimum AFIS sample size has been set empirically at  $\geq 500$  fibers or  $\geq 100$  mg per analysis (Wartelle et al., 1995), but AFIS sample size can be set anywhere between 1 and 10 000 fibers, according to fiber availability and experimental design requirements. Each AFIS sample analysis, which requires less than 5 min, produces a 19-factor data set that includes sample means of length, diameter, area, circularity, and the associated distribution percentages, that is, short fiber contents from fiber length measurements and immature fiber fraction and fine fiber fraction from

distributions of circularities and cross-sectional areas, respectively. This report, which is based on data subsets from field studies of Upland cotton and Pima cotton grown in South Carolina and Mississippi in 1992, and 1993, describes the use and potential of AFIS in generating replicated fiber-quality data and maps appropriate for studies of the complex relationships among growth environment, production practices, and fiber quality.

## MATERIALS AND METHODS

The production model AFIS length and diameter module (Zellweger-Uster, Knoxville, TN) at Southern Regional Research Center, New Orleans, LA, is augmented with a prototypic fineness and maturity module. Fiber samples, which may be dissected, hand- or machine-ginned, require no special preconditioning before AFIS analyses. The fiber count is set by the operator at 1 to 10 000 fibers, according to available sample weight. AFIS-length and diameter module determinations described here were made on 2500 fibers. Each AFIS fineness and maturity mean reported represents 10 000 fibers. Samples are pulled by hand into tufts (beards) and fed into AFIS where the fibers are separated and individualized by an internal mini-card. The individualized fibers are transported in a high-speed air stream that moves perpendicularly to a ribbon beam of light. The light blocked by an individual fiber is directly proportional to its mean optical diameter and length or time-of-flight in the sampling volume (Bragg and Shofner, 1993). The light-attenuation signal is analyzed in AFIS-length and diameter module quantitations of fiber length by number, and length by weight, and of fiber diameter by number. Short fiber contents by number and weight (percentages of fibers <12.7 mm) are generated by the AFIS-length and diameter module from the corresponding fiber length distributions.

The  $40^\circ$  light-scattering signal is analyzed in AFIS fineness and maturity module measurements of fiber cross-sectional areas by number,  $A(n)$ , and of circularity,  $\theta$  (degree of fiber wall thickening). Fiber perimeter,  $P$ , is calculated from the cross-sectional area and circularity according to the formula,  $\theta = 4\pi A(n)P^{-2}$ , in which units of  $A(n)$  and  $P$  are  $\mu\text{m}^2$  and  $\mu\text{m}$ , respectively. Immature fiber fractions are derived from the distributions of

dimensionless  $\theta$  and represent the percent of fibers for which the circularity,  $\theta < 0.25$ , when  $\theta = 1$  for a perfect circle. Fine fiber fractions are obtained from the distributions of the cross-sectional areas,  $A(n)$ , fine fiber fractions being the percent of fibers with  $A(n) < 60 \mu\text{m}^2$ . Both AFIS modules were calibrated using International Calibration Cottons (Agricultural Marketing Service, USDA, Memphis, TN). The slopes of least-squares fits of  $\mu\text{AFIS}$  and HVI micronaire values of 120 calibration samples were the same. The  $\mu\text{AFIS}$  regression line intercept was 0.3 units higher (O. Hinojosa, 1992, personal communication). AFIS fiber maturity quantities, cross-sectional area [ $A(n)$ ], circularity ( $\theta$ ), and  $\mu\text{AFIS}$ , were further evaluated through sequential AFIS fineness and maturity and x-ray fluorescence spectroscopic Ca analyses of fiber samples of known chronological maturity (Wartelle et al., 1995).

All fiber samples for AFIS analyses described here were drawn from several on-going fiber-quality X production practices field studies in South Carolina and Mississippi. Fiber-quality variability was mapped according to boll position, using 'Pee Dee 3', an Upland cotton genotype grown in South Carolina on Eunola loamy sand (fine-loamy, siliceous, thermic Aquic Hapludults). All sympodial branch flowers on Pee Dee 3 plants in 1 m of row were tagged five times a week at 0 d post anthesis from 16 July to 31 Aug. 1992. Just prior to harvest, tagged plants were removed and fruiting-site maps of each plant were made. A node was that place on the main stem where a fruiting branch (sympodium) arose. Node 0 was the cotyledonary node (Bradow et al., 1997a). Position represented the order in which buds were produced on a sympodium. Fruiting site was a specific node-position combination. Four bolls from each fruiting site were randomly selected for AFIS analyses. After boll and locule weights were determined, individual locules were ginned separately by a reciprocating-knife roller gin. All fibers from an individual locule constituted one AFIS sample and one replicate for statistical analysis ( $n = 12$  intact locules without disease symptoms).

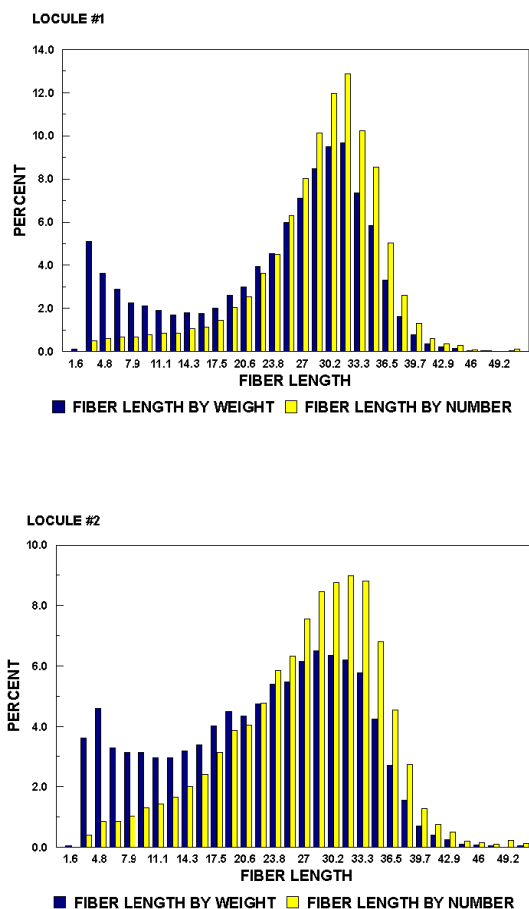
The genotypes in the fiber maturation rate study were 'DES119', an Upland cotton and 'Pima S-6' grown in 1992 and an Upland genotype, 'Deltapine 5415' (DPL5415), and Pima S-6 grown in 1993 in Mississippi on a Marietta sandy clay loam (fine-

loamy, siliceous, thermic Fluvaquentic Eutrochrepts). Bolls were harvested at 21, 28, 35, 42, or 56 d post anthesis. Bracts and stems were removed from the bolls before fresh weights were recorded, and the bolls were cut open and frozen thoroughly before dissection. Bolls were freeze-dried. Each boll was carefully separated into burr, lint, and seeds. The lint fibers from each individual boll were analyzed sequentially by AFIS and Ca x-ray fluorescence. All fiber from a single boll represented one statistical replication [ $n = 6$  bolls].

All AFIS fiber quality data were analyzed as completely random two-way (days post anthesis by genotype or node by position in the case of Pee Dee 3) factorial designs (Sokal and Rohlf, 1981; MSTAT-C. 1991. MSTAT Microcomputer Statistical Program, Michigan State Univ., East Lansing). Means were separated after additional one-way analyses of variance by Tukey's least significant difference testing.

## RESULTS AND DISCUSSION

Fiber length is considered the premier fiber quality because staple length is closely correlated with processing efficiency and the quality of the yarn produced (Perkins et al., 1984). In research situations, detailed length information, such as frequency distributions of fiber length by weight or length by number, has been obtained by time- and labor-intensive sorting and weighing methods, for example, the Suter-Webb (Behery, 1993). During USDA cotton classing, length and length uniformity determinations by HVI (Method D 4604-86 [ASTM, 1988]) are accomplished by pneumatically scanning a clamped combed fiber sample (test beard). The HVI length analyzer measures the air pressure drop across an orifice as the fiber sample is passed through the orifice. The pressure drop across the orifice is proportional to the total specific area of fibers in the orifice at any time as the test beard is moved through the orifice. It is assumed that each fiber in the test beard is caught in the specimen clamp in proportion to its length, compared to the total length of all fibers in the sample, and that the clamp point on a fiber is random along its length. Length determinations by the HVI of ASTM Method D 4604-86 (ASTM, 1988) require a minimum 3 to 3.3 g of fiber and represent a relatively rapid method for determining



**Fig. 1. Distributions of length by number,  $L(n)$ , and length by weight,  $L(w)$ , in fiber from two randomly chosen Pee Dee 3 locules at fruiting site, node 9, position 2. Each sample contained 2500 fibers.**

fiber length characteristics of composite or bulk cotton samples.

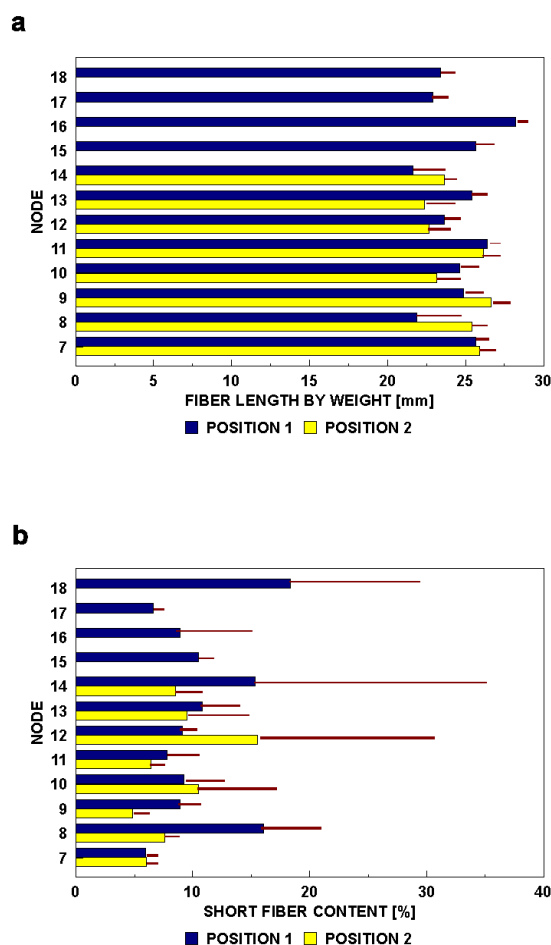
Length data from HVI, due to the effects of sample pooling, fiber crimp, specimen clamp characteristics, and other factors, do not necessarily agree with data obtained by other fiber length determination methods (Behery, 1993). However, excellent correlations have been found between fiber length measurements made by AFIS-length and diameter module, the Suter-Webb comb sorter, and the Peyer Texlab Almeter, an electronic mass-sensing device (references cited in Behery, 1993).

Minimum sample-size requirements for all fiber length measurement methods, except the AFIS-length and diameter module, significantly exceed the approximately 0.5 g of fiber available from a normal, mature cotton locule (Jenkins et al., 1990). In less than 6 min, AFIS generated the mean fiber

length by number, and the weight-biased fiber length by weight means, for two Pee Dee 3 locules from a randomly selected Pee Dee 3 boll from node 9, position 2 (Fig. 1). The number of fibers assayed was 2500 per locule. Mean length by number and length by weight of locule-1 were  $23.4 \pm 10.6$  mm and  $27.7 \pm 7.1$  mm, respectively. The length by number and length by weight means of locule-2 were  $21.3 \pm 10.2$  mm and  $26.2 \pm 8.2$  mm.

In determinations of means and distributions of fiber lengths by number, the cross-sections and weights of the individual fibers are not considered. Each fiber length is incorporated according to its numerical frequency. A distribution or mean of fiber lengths by weight, is weight-biased so that the fibers are incorporated in the computation according to their weight. A length by weight distribution is characterized by a lower incidence of short fibers, and mean length by weight is always greater than mean length by number for a given sample. Independent of fruiting site, 1992 Pee Dee 3 length by weight means averaged  $21.7 \pm 4.1\%$  higher than the corresponding length by number means.

The frequency distribution shapes in Figure 1 demonstrate the marked differences in fiber length within and between individual Pee Dee 3 locules and, therefore, within and among Pee Dee 3 bolls. The major component of this natural variance is the individual seed (Behery, 1993; Davidonis and Hinojosa, 1994). Pee Dee 3 Length variability was also significant when mean fiber lengths by weight, from positions 1 and 2 at node 7 through node 14 and position 1 at node 15 through node 18 were compared (Fig. 2a). (Too few position 2 bolls were found above node 14 to allow valid statistical comparisons.) The maximum mean length by weight of  $28.2 \pm 0.3$  mm occurred at node 16, position 1; the length by weight minimum was  $21.6 \pm 0.2$  mm at node 14, position 1. The overall Pee Dee 3 mean length by weight was  $24.5 \pm 1.8$  mm. Commercial fibrograph measurements (2.5% span length,  $n = 8$ ) indicated a bulk 1992 Pee Dee 3 fiber length of  $29.7 \pm 0.5$  mm. Span length is the distance spanned by a specific percentage of fibers in the sample test beard (ASTM D 1447-89, 1994). The AFIS-length and diameter module length by weight means for 56 d-post-anthesis Mississippi-grown genotypes were DPL5415,  $25.4 \pm 0.5$  mm; DES119,  $23.9 \pm 3.0$  mm; and Pima S-6,  $27.7 \pm 2.3$  mm in



**Fig. 2.** Mean Pee Dee 3 fiber lengths by weight,  $L(w)$ , and short fiber contents [ $SFC(w)$  or percentage of fiber of  $L(w) < 12.7$  mm] at positions 1 and 2 between nodes 7 and 18. (Means and standard errors, single lines to right of bars, represent 2500 fibers each from 12 locules in four randomly selected bolls from each fruiting site.) Fig. 2. Mean Pee Dee 3 fiber lengths by weight,  $L(w)$ , and short fiber contents [ $SFC(w)$  or percentage of fiber of  $L(w) < 12.7$  mm] at positions 1 and 2 between nodes 7 and 18. (Means and standard errors, single lines to right of bars, represent 2500 fibers each from 12 locules in four randomly selected bolls from each fruiting site.)

1992 and  $28.9 \pm 0.5$  mm in 1993. Length uniformity was significantly lower in the 1992 DES119 and Pima S-6 crops. Weights of fibers in the Mississippi maturation study, particularly those from bolls harvested before 42 d post anthesis, were too low for commercial length determinations

Mean fiber lengths, however, gave no indication of the short fiber content by weight, a major concern for textile manufacturers because elevated numbers of short fibers represent a significant waste component and short fiber content

is an important processing-quality factor (Deussen, 1992; Behery, 1993; Deussen and Faerber, 1995). Short fiber content by weight and short fiber content by number represent the percent of fibers, by weight or number, with lengths  $\leq 12.7$  mm. In Figure 2b, the minimum length by weight, at node 14, position 1 corresponded to a maximum short fiber content by weight mean of  $16.0 \pm 18.0\%$ , the combination of high short fiber content by weight mean and standard deviation being an indicator of variable growth and development of the bolls at that node and branch position (Bradow et al., 1997b). The high short fiber content by weight means at node 12, position 2 and node 18, position 1 had standard deviations  $>14.0$  percentage points. However, the equally high short fiber content by weight at node 8, position 1 was  $16.1 \pm 6.0\%$ . Elevated short fiber content was not always associated with increased fiber length variation, and the level of variability did not correlate with boll position. Mean 1992 Pee Dee 3 short fiber content by weight was  $9.8 \pm 3.6\%$ .

Within the global textile industry there is growing concern over waste and decreased fiber value related to increased short fiber content, but short fiber content is not yet included in USDA cotton classing information. Since HVI can provide short fiber content as one of the classed fiber qualities and short fiber content has a significant technical effect on fiber processing, yarn properties, and fabric performance, establishment of premiums and discounts on the basis of short fiber content is anticipated (Deussen, 1992; Behery, 1993; Deussen and Faerber, 1995). Thus, breeders, producers, and processors will all benefit from improvements in genotype characteristics and production practices that decrease short fiber content and fiber length variability.

Unlike, short fiber content and fiber length, specific processing qualities have yet to be definitely linked to fiber diameter, the other fiber quality measured by the AFIS-length and diameter module. Significant diameter variations [ $P < 0.0001$ ] occurred within locules and among fruiting sites in Pee Dee 3. Mean fiber diameter in locule 1 from node 9, position 2 was  $13.6 \pm 4.5 \mu\text{m}$ , compared to  $11.8 \pm 5.4 \mu\text{m}$  in locule 2. The diameter distributions by locule and fruiting site are shown in Figure 3. The greatest fiber diameters were found at node 10, position 2 and node 17,

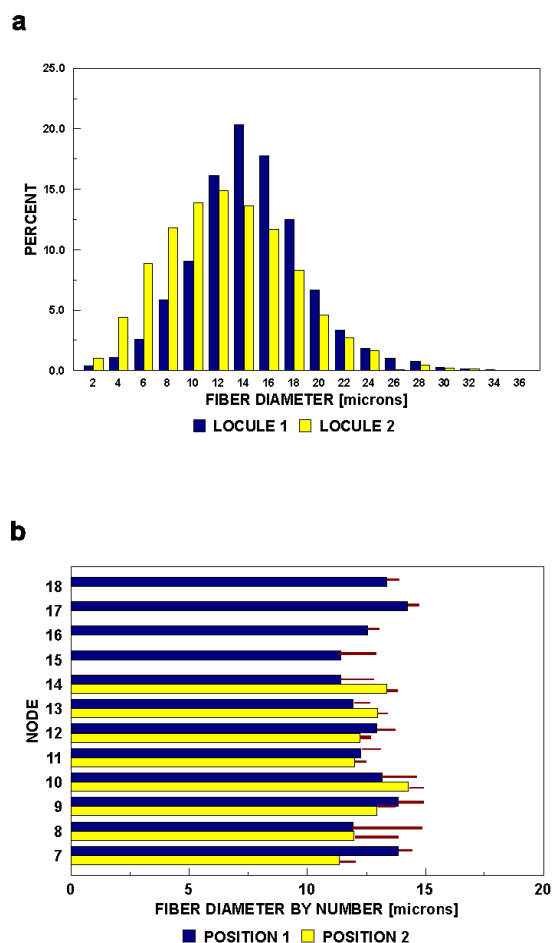


Fig. 3. (a) Mean Pee Dee 3 fiber diameters by number,  $D(n)$ , from two randomly chosen Pee Dee 3 locules at node 9, position 2. (b) Distribution of Pee Dee 3 fiber  $D(n)$  according to fruiting site. (Means and standard errors, single lines to right of bars, represent 2500 fibers each from 12 locules in four randomly selected bolls from each fruiting site.)

position 1. Fibers with the smallest diameter were found at node 7, position 2. The overall average Pee Dee 3 fiber diameter was  $12.7 \pm 0.9 \mu\text{m}$ . Distributions of fiber diameters among fruiting site were unrelated to both the length by weight and the length by number distributions among fruiting sites, although fiber diameter has sometimes been considered a measure of biological fineness and a genotype characteristic linked to staple length (Ramey, 1982). The AFIS length and diameter module diameters of Mississippi-grown Upland cotton fibers at 56 d post anthesis ranged from  $15.5 \pm 0.3 \mu\text{m}$  for DPL5415 to  $14.3 \pm 1.1 \mu\text{m}$  for DES119. The genetically finer fibers of Pima S-6 had diameters of  $10.27 \pm 0.6$  to  $11.1 \pm 0.9 \mu\text{m}$  at the

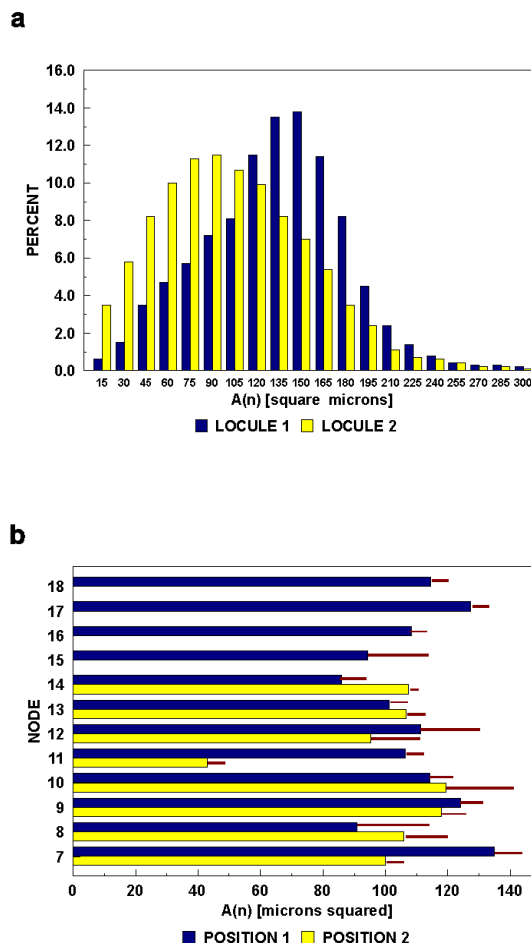
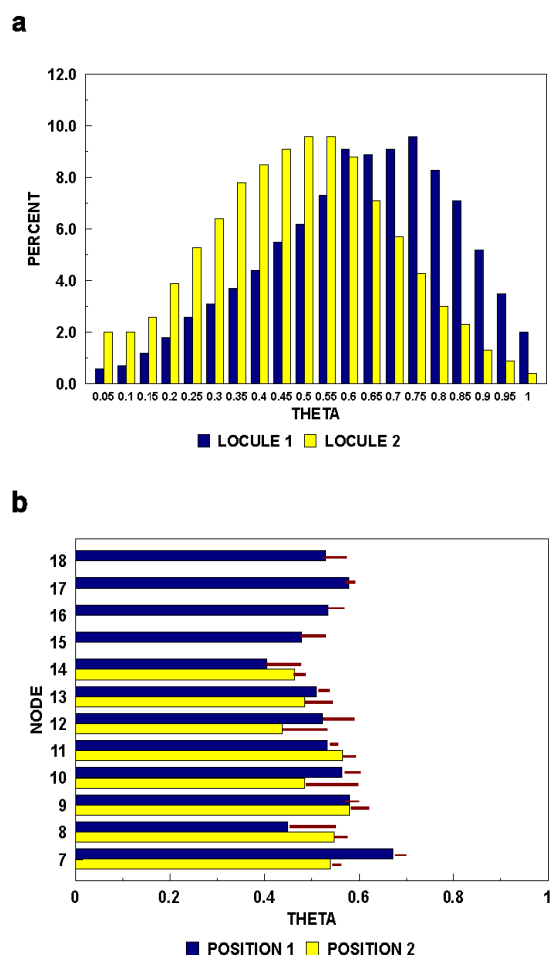


Fig. 4. (a) Mean Pee Dee 3 fiber cross-sectional areas,  $A(n)$ , from two randomly chosen Pee Dee 3 locules at node 9, position 2. (b) Distribution of Pee Dee 3 fiber  $A(n)$  according to fruiting site. (Means and standard errors, single lines to right of bars, represent 10 000 fibers each from 12 locules in four randomly selected bolls from each fruiting site.)

same stage of chronological maturity.

Closely related to the AFIS length and diameter module fiber diameters are the fiber cross-sectional areas and circularities quantified by the AFIS fineness and maturity module. Circularity ( $\theta$ , degree of fiber wall thickening) is the ratio of the cross-sectional area and square of the perimeter,  $P$ , [ $\theta = 4\pi A(n)P^{-2}$ ]. During the final stages of boll maturation and opening, cotton fibers collapse into a variety of flat, bean, or horseshoe cross-sections, the degree of collapse from circular being dependent on thickness of the cellulosic secondary cell wall of the fiber. Fiber wall thickness is a major factor in fiber processing because high ratios of thin-walled, immature fibers significantly increase



**Fig. 5.** (a) Mean Pee Dee 3 fiber circularities,  $\theta$ , from two randomly chosen Pee Dee 3 locules at node 9, position 2. (b) Distribution of Pee Dee 3 fiber  $\theta$  according to fruiting site. (Means and standard errors, single lines to right of bars, represent 10 000 fibers each from 12 locules in four randomly selected bolls from each fruiting site.)

the number of spinning, yarn, and dye defects (Ramey, 1982). The ideal, mature cotton fiber has a fully developed secondary wall that fills the cell lumen without increasing the cross-section. Fine, but mature, fibers have more desirable yarn strength and spinning and dye uptake properties.

The AFIS fineness and maturity module quantifies fiber fineness as mean cross-sectional area by number and fine fiber fraction, the percentage of fibers of  $A(n) < 60 \mu\text{m}^2$ . The distribution and number of fine fibers differed in the two Pee Dee 3 locules from node 9, position 2 (Fig. 4a). The lower short fiber content by weight in locule 1 (5.0%) corresponded to a mean fiber cross-section of  $125.1 \pm 47.6 \mu\text{m}^2$ . In locule 2, short fiber

content by weight was 7.6%, and cross-sectional area was  $94.9 \pm 51.1 \mu\text{m}^2$ . The corresponding fine fiber fraction percents were 10.3% for locule 1 and 27.3% for locule 2. The variability found between locules was also evident among nodes and positions (Fig. 4b). The maximum mean cross-sectional area, of  $135.0 \pm 4.0 \mu\text{m}^2$  occurred at node 7, position 1; minimum  $A(n)$ ,  $85.9 \pm 3.8 \mu\text{m}^2$ , occurred at node 14, position 1. The corresponding fine fiber fraction means were  $6.3 \pm 1.4\%$  and  $33.8 \pm 19.5\%$ . Overall mean cross sectional area,  $A(n)$ , was  $107.1 \pm 20.2 \mu\text{m}^2$ , and composite fine fiber fraction was  $17.9 \pm 11.5\%$ .

The HVI does not measure fiber cross-sectional area per se, and fineness of cotton fibers, like yarn fineness, is expressed in gravimetric terms as the linear density or weight per unit length, usually fiber millitex, that is,  $\mu\text{g m}^{-1}$  (Munro, 1987). Direct measurements of gravimetric fineness are time-consuming and of limited usefulness without accompanying microscopic measurements of biological fineness, that is, fiber diameters (Ramey, 1982). Direct measurements of biological fiber fineness are quite costly in time required for each assessment and are strongly biased by sampling errors and the natural, highly convoluted, noncircular shape of cotton fibers. During cotton classing, fiber fineness is measured indirectly as micronaire, an air-permeability parameter that estimates fiber surface area (Ramey, 1982; Munro, 1987). However, micronaire is significantly affected by fiber physical maturity, that is, the relative cell wall thickness of the fibers (Perkins et al., 1984).

The AFIS fineness and maturity module provides a discrete measure of fiber physical maturity in the form of mean circularity and immature fiber fraction, the percentage of fiber with  $\theta < 0.25$ . The Pee Dee 3 locule 1 fibers with the larger cross-sections also had the greater wall thicknesses [Fig. 5a]. Mean  $\theta$  (circularity) for 10 000 fibers from locule 1, node 9, position 2 was  $0.599 \pm 0.209$ , which corresponded to an immature fiber fraction of 6.9%. In locule 2,  $\theta$  was  $0.461 \pm 0.201$ , and immature fiber fraction was 15.71%. Variations in fiber wall thickness were also seen among Pee Dee 3 fruiting site (Fig. 5b). The maximum circularity,  $\theta$ , of  $0.671 \pm 0.030$  occurred at node 7, position 1; minimum  $\theta$ ,  $0.404 \pm 0.070$ , was found at node 14, position 1. These were the same fruiting sites at which the maximum and



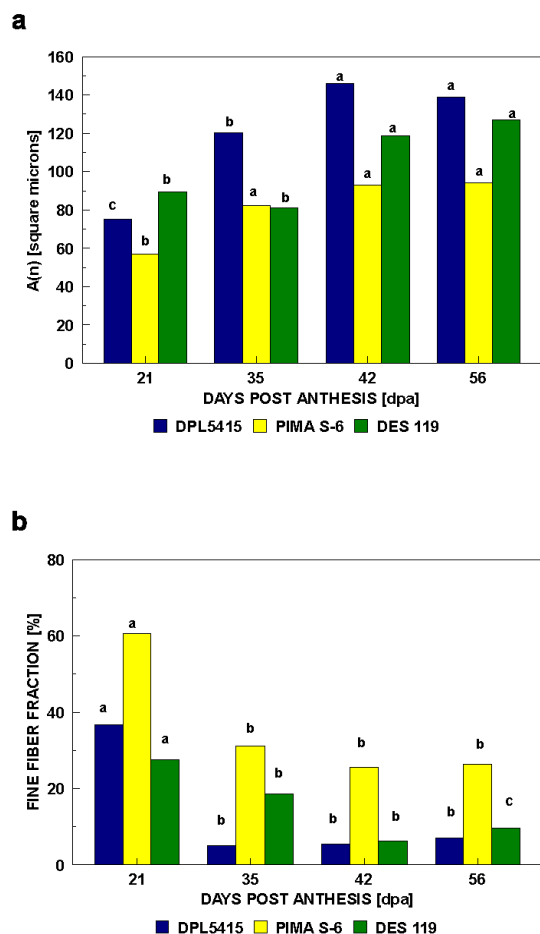


Fig. 6. Mean cross-sectional areas,  $A(n)$ , and fine fiber fractions [FFF or percent fibers with  $A(n) \leq 60 \mu\text{m}^2$ ] of DPL5415, DES119, and Pima S-6 fibers at 21, 35, 42, and 56 d post-floral anthesis (dpa). Genotype means associated with the same letter are not significantly different ( $P = 0.001$ ).

minimum cross-sectional area,  $A(n)$ , means were found. Maximum and minimum immature fiber fractions were 26.2 and 4.5%, respectively. The overall circularity,  $\theta$ , mean was  $0.521 \pm 0.090$ , corresponding to immature fiber fraction of 12.9  $\pm$  9.2%.

The AFIS fineness and maturity module capacity for separately quantifying the fineness and wall-thickness components of fiber physical maturity is particularly useful in studies of fiber maturation. When cross-sectional areas were obtained for DPL5415, DES119, and Pima S-6 fibers of differing chronological maturities (21, 35, 42, or 56 d post anthesis), genotype differences in fiber fineness and maturation rates were apparent (Fig. 6a). In 1993, DPL5415 cross-sectional area

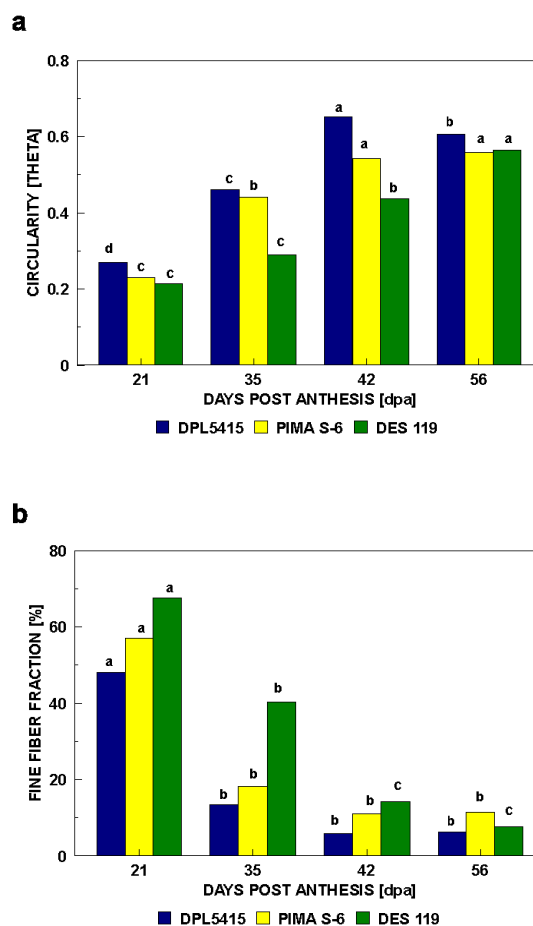


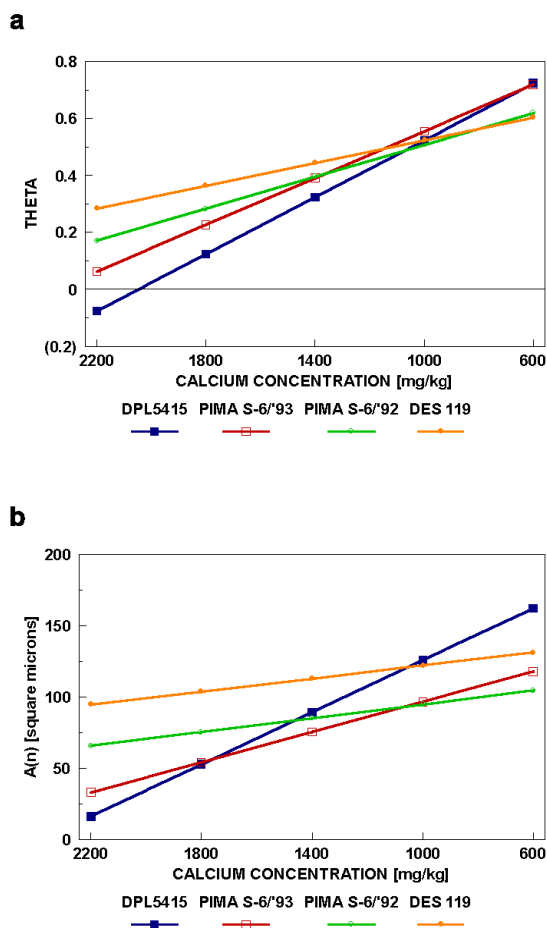
Fig. 7. Mean fiber circularities,  $\theta$ , and immature fiber fractions (IFF or percent of fibers with  $\theta \leq 0.25$ ), of DPL5415, DES119, and Pima S-6 fibers at 21, 35, 42, and 56 d post-floral anthesis (dpa). Genotype means associated with the same letter are not significantly different ( $P = 0.001$ ).

increased according to the rate equation, DPL5415  $A(n) = (62.7 + 1.5 \times \text{d post anthesis } \mu\text{m}^2)$ ,  $r^2 = 0.664$ . The lower rate (slope) in the corresponding equation for DES119 grown in 1992, DES119  $A(n) = (64.9 + 1.1 \times \text{d post anthesis } \mu\text{m}^2)$ ,  $r^2 = 0.640$ , has been attributed to suboptimal temperatures early in the 1992 boll- and fiber-filling period (Bradow et al., 1995). The rate equations for Pima S-6 were 1993 Pima S-6  $A(n) = (47.0 + 0.9 \times \text{d post anthesis } \mu\text{m}^2)$ ,  $r^2 = 0.671$ ; and 1992 Pima S-6  $A(n) = (54.1 + 0.8 \times \text{d post anthesis } \mu\text{m}^2)$ ,  $r^2 = 0.712$ . The lower intercepts and slopes of Pima S-6 maturation rate plots reflect the greater biological fineness of *G. barbadense*. The linear components of all regressions were significant ( $P < 0.0001$ ), and the majority of the corresponding quadratic components

were not significant. These and subsequent maturation rate equations are offered as examples of the uses and potential of the AFIS fineness and maturity module. Full analyses and discussions of the complete multi-year Mississippi and South Carolina field studies will appear in subsequent reports.

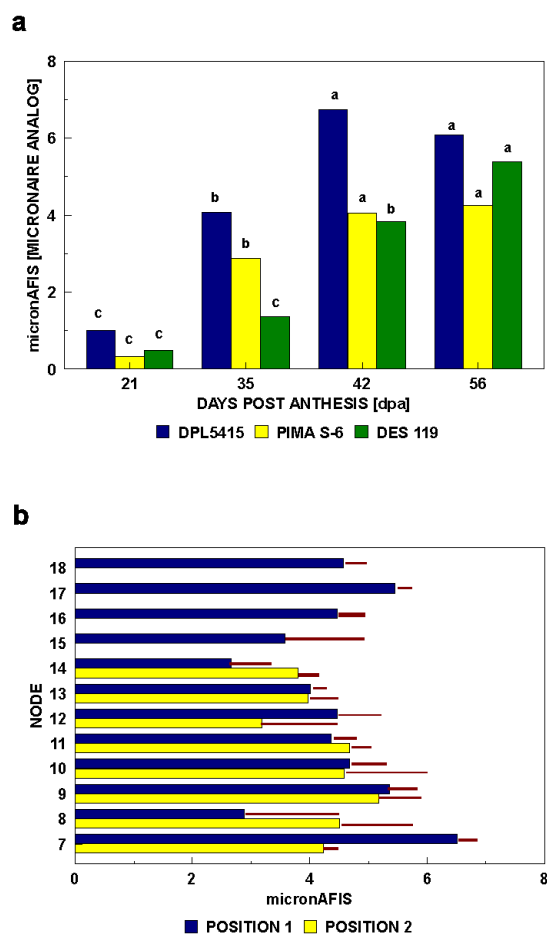
Fiber wall thickening during maturation was also quantified between 21 and 56 d post anthesis (Fig. 7a). Again, the relationships between fiber physical maturity, (circularity or  $\theta$ ) and chronological maturity had strong linear components ( $P < 0.0001$ ). The Upland cotton wall thickening rates were described by DPL5415  $\theta = (0.173 + 0.006 \times d \text{ post anthesis})$ ,  $r^2 = 0.736$ ; and DES119  $\theta = (0.053 + 0.009 \times d \text{ post anthesis})$ ,  $r^2 = 0.867$ . The Pima S-6 wall thickening rates were described by 1993 Pima S-6  $\theta = (0.127 + 0.008 \times d \text{ post anthesis})$ ,  $r^2 = 0.875$ ; and 1992 Pima S-6  $\theta = (0.023 + 0.009 \times d \text{ post anthesis})$ ,  $r^2 = 0.916$ . In Figure 7b, immature fiber fraction means of all three genotypes were  $<15\%$  at 42 d post anthesis. The highest 56-d post anthesis immature fiber fraction (11.5%) was that of Pima S-6, an effect due in part to the calibration of the AFIS fineness and maturity module with Upland cottons (Fig. 7b). The 56-d post anthesis immature fiber fraction means of DPL5415 and DES119 were 6.3 and 7.7%, respectively.

Both circularity and cross-sectional area quantified fiber physical maturity by measuring secondary wall deposition over time during fiber maturation. Good correlations were found between secondary wall deposition rates measured as AFIS fineness and maturity module circularity, and the rates of secondary wall cellulose deposition quantified by x-ray fluorescence determinations of the relative dilution by weight over time of primary wall Ca (Wartelle, et al., 1995). When DPL5415 and DES119  $\theta$  values were regressed on Ca concentrations, close linear relationships ( $r^2 \geq 0.843$ ) were found between fiber physical maturity measured as AFIS fineness and maturity module  $\theta$  and Ca weight ratio quantitation of biochemical maturity of the same fiber samples. Ca weight ratio quantitation of biochemical maturity was less close and linear [ $r^2 = 0.411$  in 1993, and  $r^2 = 0.778$  in 1992]. The relationship between DPL5415 cross-sectional area and Ca weight ratio quantitation by x-ray fluorescence maturity estimates was also linear



**Fig. 8.** Cotton fiber maturation rates calculated from regressions on cell wall Ca ratios by weight (Ca weight ratio quantitation by x-ray fluorescence, Ca-XRF levels) at 21, 28, 35, 42, and 56 d post anthesis of (a) fiber circularities,  $\theta$ , or (b) cross-sectional areas,  $A(n)$ , of cotton genotypes, DPL 5415 and Pima S-6 grown in 1993 and DES 119 Pima S-6 grown in 1992. [Based on means of AFIS-fineness and maturity and Ca-XRF maturity estimates ( $n = 6$ ).]

[ $r^2 = 0.895$ ]. The effects of suboptimal temperatures in 1992 on DES119 fiber maturation (Fig. 8b) may have decreased the correlation ( $r^2 = 0.567$ ) between DES119 cross-sectional area and Ca weight ratios determined by x-ray fluorescence spectroscopy (Bradow et al., 1995). Compared to the Upland genotype, DPL5415, the Pima S-6 physical maturity parameter, cross-sectional area, was less closely correlated with the Ca weight ratios determined by x-ray fluorescence biochemical maturity estimate (1992 Pima S-6  $r^2 = 0.638$ , and 1993 Pima S-6  $r^2 = 0.494$ ). All relationships between AFIS fineness and maturity module physical maturity and Ca weight ratios determined by x-ray fluorescence



**Fig. 9.** (a) Mean  $\mu$ AFIS of DPL5415, DES119, and Pima S-6 fibers at 21, 35, 42, and 56 d post-floral anthesis (dpa) for which genotype means associated with the same letter are not significantly different,  $P = 0.001$ ; (b) distribution of PD3 fiber  $\mu$ AFIS according to fruiting site. (Means and standard errors, single lines to right of bars, represent 10 000 fibers each from 16 locules in four randomly selected bolls from each fruiting position.)

biochemical maturity quantitations were best described by linear regression equations.

Although micronaire is known to measure fiber surface area and to be affected by both fiber fineness and wall thickness, this airflow resistance method is the standard instrumental measure of fiber maturity (Ramey, 1982; Perkins et al., 1984; Lord and Heap, 1988). The AFIS fineness and maturity module calculates a micronaire analog,  $\mu$ AFIS, from each sample data set. In Figure 9a are shown the increases over time in  $\mu$ AFIS of DPL5415, DES119, and Pima S-6. The AFIS fineness and maturity module maturation rates of the Upland genotypes were linear [ $P > 0.0001$ ] and best described by the equations DPL5415  $\mu$ AFIS =

(0.124 x d post anthesis - 0.335),  $r^2 = 0.721$ ; DES119  $\mu$ AFIS = (0.125 x d post anthesis - 1.775),  $r^2 = 0.828$ . The 'best-fit' *G. barbadense* equations were 1992 Pima S-6  $\mu$ AFIS = (0.108 x d post anthesis - 1.930),  $r^2 = 0.897$  and 1993 Pima S-6  $\mu$ AFIS = (0.100 x d post anthesis - 0.892),  $r^2 = 0.753$ . Genotype effects on slope noted in cross-sectional area and circularity data were minimal in fiber maturation rates based on  $\mu$ AFIS. All regressions were linear and significant ( $P = 0.0001$ ).

Fiber-maturity differences between Pee Dee 3 locules reported above were also seen in  $\mu$ AFIS values of locule 1 ( $\mu$ AFIS = 5.6) and locule 2 ( $\mu$ AFIS = 3.4). The variations in Pee Dee 3  $\mu$ AFIS by node and position are shown in Figure 9b. Maximum Pee Dee 3  $\mu$ AFIS was  $6.5 \pm 0.4$  and occurred at node 7, position 1. The corresponding minimum was  $2.7 \pm 0.9$  at node 14, position 1. Overall composite mean  $\mu$ AFIS at all Pee Dee 3 fruiting sites was  $4.4 \pm 1.3$ , and the mean of four commercial bulk micronaire determinations for this 1992 Pee Dee 3 crop was  $4.2 \pm 0.2$ . Both the composite  $\mu$ AFIS and bulk micronaire values fall within the premium-price micronaire range, 3.5 to 4.9, for Upland cotton. However, Figure 9b clearly demonstrates that those bulk estimates of fiber maturity conceal the frequency and range of fiber maturity variations falling well outside the bulk premium micronaire values.

Commercial fiber-maturity testing instrumentation was developed for rapid micronaire determinations of large, bulk fiber samples, that is, samples taken for classing purposes from each bale after ginning. The small-sample multi-factor quantitations by the AFIS fineness and maturity module made possible the replicated per-boll "micronaire" estimates in Figure 9 and the examination, on a locule by locule basis, of fineness and wall thickness, important components of fiber physical maturity that are estimated in combination by other fiber maturity tests. The small-sample quantitation capabilities of AFIS were also invaluable for mapping other fiber shape, size, and physical maturity parameters by fruiting site (Figures 2 through 5).

No commercial and few experimental (Sequeira et al., 1994) cotton whole-plant simulations include fiber quality measured on an organ-by-organ or fruiting site basis. Multiple replications of

simultaneous estimates of fiber shape maturity from a single small sample allow valid statistical comparisons among fruiting site or locules and between plants and/or treatments, for example, irrigation (Bradow et al., 1994). Replicated quantitations of fiber quality, like those obtained with AFIS, are fundamental to the creation of statistical models that simulate and predict the effects of the environment and crop management practices on cotton fiber quality and processing success. The rapid, small-sample analyses possible with AFIS also provide a powerful tool for filling the information gap between the yield data provided by whole-plant models and the predictive fiber quality data needed by the textile industry.

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