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Applications of AFIS Fineness and Maturity Module and X-Ray Fluorescence Spectroscopy in Fiber Maturity Evaluation

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ABSTRACT

Worldwide round-testing and calibration of the nep-counting and fiber-length modules of the Zellweger Uster advanced fiber information system (AFIS) are well advanced. Lack of appropriate quantitative calibration standards for fiber maturity has limited similar development of the prototypic AFIS fineness and maturity (F&M) module. A combination of calcium x-ray fluorescence spectroscopy (Ca-XRF) and AFIS-F&M mapping of fiber quality from twenty-one days post anthesis (DPA) to boll opening (56 DPA) permits direct comparisons of AFIS-determined fiber physical maturity (as micronAFIS, circularity, and cross-sectional area) with fiber chronological maturity (as DPA) and physiochemical maturity (as Ca-XRF). The AFIS-F&M module is a powerful tool that makes possible quantitative comparisons of fiber maturity across time (during fiber development and in different crop years), across space (different boll and locule positions and different growing areas), within single bolls and locules, and between cotton varieties and species.

In the U.S., price, marketability, and utility value of cotton lint are determined during cotton classing [14]. The high volume instrumentation (HVI) system used in U.S. classing offices provides quantitations of fiber parameters, *i.e.*, micronaire, fineness, length and length uniformity index, short fiber content, strength and elongation, and maturity, as well as instrumental readings of color, trash, and sugar content [3, 9, 11]. Standard HVI fiber shape and size measurements require a minimum of 3.0 to 3.3 grams of randomly collected, ginned fibers subjected before testing to defined pretreatment or conditioning [1]. The HVI sample weight requirement significantly exceeds single-boll fiber weight, thus precluding examination of fiber quality levels and uniformity at the boll, locule, or seed level. Since the major component of fiber quality variance is at the seed level [2], rapid, reproducible, statistically valid, small-sample quantitations of fiber quality are essential if fiber quality levels and uniformity are to be improved.

The sample size for fiber quality determinations with the Zellweger-Uster advanced fiber information system (AFIS)[1] equipped with the prototypic fineness and maturity (F&M) module can theoretically be set between 1 and 10,000 fibers, depending on available fiber sample size. However, a practical AFIS-F&M sample-size lower limit has been set empirically at >500 fibers or 100 mg fiber [13]. This small-sample capability of AFIS-F&M is particularly valuable in research situations where fiber quality (size and shape) measurements must be made on a per seed, locule, or boll basis.

The Zellweger-Uster (Knoxville, TN) AFIS-F&M is an airflow, electro-optical particle sizer that rapidly and reproducibly quantifies fiber circularity (θ or degree of fiber wall thickening) and cross-sectional area by number $A(n)$ by analyzing the light scattered at a 40° angle

[1] 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 of service to the exclusion of others that may be suitable.

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as fibers flow perpendicularly to the light beam [2, 5]. The relationship between θ, $A(n)$, and the calculated fiber perimeter P is expressed by the formula $\theta = 4\pi A(n)/P^2$. Immature fiber fraction (IFF) is derived from the distribution of θ, and is defined as the percent of fibers of $\theta < 0.25$. Fine fiber fraction (FFF) is obtained from the distribution of $A(n)$ and represents the percent of fibers of $A(n) < 60\ \mu m^2$. A micronaire analog, micronAFIS, is also calculated using the AFIS-F&M. The corresponding fiber lengths by both number and weight as well as fiber diameters, are available from the AFIS-L&D (length and diameter) module, which also generates short fiber contents (% of fiber lengths < 0.5 inches) from the fiber length distributions.

The AFIS, like HVI, can be calibrated with "check cottons" such as standard AMS calibration cottons or through interlaboratory round-testing. Recently, a physiochemical fiber maturity test, Ca-XRF calibrated against NIST (National Institute of Standards and Technology) standards [13], provided AFIS-F&M corroboration when fibers dissected from bolls of known chronological maturity were analyzed sequentially by AFIS-F&M followed by Ca-XRF. The AFIS-F&M module provided micronAFIS maturity estimates of fiber samples > 500 fibers or >100 mg and separately quantified the cross-sectional (fineness) and wall-thickening (physiological maturity) components of fiber physical maturity. The AFIS-F&M plus Ca-XRF sequence was also used to quantify the fiber maturity components from long-fibered motes [4, 6, 7, 8]. Alone, AFIS-F&M has been used to map fiber maturity (*i.e.*, $A(n)$, θ, and micronAFIS) according to boll position at the locule level [3].

In this paper, we describe the quantitation by AFIS-F&M and Ca-XRF of physical and physiochemical maturity of Upland and Pima cotton fibers of known chronological age. We also discuss the uses of AFIS-F&M in following quantitative changes in fiber quality during maturation from 21 to 56 DPA and in mapping fiber maturity according to boll position or seed size.

Materials and Methods

COTTON VARIETIES, CULTURE, AND FIBER SAMPLING

The cotton genotypes analyzed by sequential AFIS-F&M and Ca-XRF in the fiber maturation rate study included the Upland (*Gossypium hirsutum*) varieties, DES119 and DPL5415, and a *G. barbadense,* Pima S-6, all grown in Mississippi during 1992 (DES119 and Pima S-6) or 1993 (DPL5415 and Pima S-6). Flower opening and boll harvest dates are shown in Table I. Bracts and stems were removed from the bolls, and the bolls were cut open and frozen thoroughly before lint was separated from seed by dissection. All fibers from each individual boll were analyzed together and sequentially by AFIS-F&M and Ca-XRF; fibers from a single boll represented one statistical replication ($n = 6$ for each genotype × year × harvest age × flowering date combination).

The long-fibered motes, subnormal-weight seed structures bearing normal length (24.6 ± 0.8 mm) fi-

TABLE I. Flower tagging and boll harvesting dates in chronological maturity study.

		Flower tagging and boll harvest dates		
Genotype	Crop year	Flowering date	Harvest date	Days post anthesis
DES119	1992	23 July	6 August	14 DPA
			13 August	21 DPA
			20 August	28 DPA
			27 August	35 DPA
			17 September	56 DPA
Pima S-6	1992	23 July	6 August	14 DPA
			13 August	21 DPA
			17 September	56 DPA
DPL5415	1993	28 July	19 August	21 DPA
			26 August	28 DPA
			9 September	42 DPA
			23 September	56 DPA
DPL5415	1993	19 August	9 September	21 DPA
			13 October	56 DPA
Pima S-6	1993	28 July	19 August	21 DPA
			26 August	28 DPA
			9 September	42 DPA
			23 September	56 DPA
Pima S-6	1993	19 August	9 September	21 DPA
			13 October	56 DPA

bers, were collected from three locule regions, *i.e.*, apical (positions 1 to 3), medial (positions 4 to 6 or 7), and basal (positions 7 or 8 to 10, the lowest two seed positions in the locule) of DPL51 bolls grown in 1992 in Corpus Christi, TX. Mote weights, including fiber, fell into three categories: 7 to 20 mg, 21 to 35 mg, and 35 to 59 mg. Normal mean DPL51 seed-cotton weight was 147.1 ± 9.3 mg per seed. Fibers from motes and normal seeds were finger-ginned before being subjected to sequential AFIS-F&M and Ca-XRF analyses.

Plant mapping studies of fiber maturity and variability distributions according to boll position used Pee Dee 3 (PD3), an Upland cotton variety grown in South Carolina during 1992. All sympodial (main) branch flowers on plants in 1-m row lengths were tagged five times a week at floral anthesis from July 16 to August 31, 1992. Just prior to harvest, the tagged plants were removed and fruiting site (boll position) maps of each plant were constructed. Fruiting site is a specific combination of node (branch) and position on the branch. Four bolls were randomly selected from bolls at each node-position combination at nodes 7 through 14 and positions 1 and 2, as well as position 1 bolls from nodes 15 to 18. Because the number of locules per boll varied and some locules were absent, we drew 12 locules at random from each node-position subset for AFIS analysis. Individual PD3 locules were ginned separately in a reciprocating-knife roller gin, and all fibers from an individual locule constituted one AFIS sample and one replicate for statistical analysis ($n = 12$).

FIBER MATURITY ANALYSES

AFIS-F&M Analyses

The production model AFIS-L&D (advanced fiber information system—length and diameter) at Southern Regional Research Center is augmented with a prototypic fineness and maturity (F&M) module (Zellweger-Uster, Knoxville, TN). Fiber samples, 0.2 gm tufts that require no special preconditioning for AFIS-F&M analysis, were drawn by hand into 25-cm slivers that were then separated and aligned by the AFIS internal mini-card into individualized fiber arrays. During analysis, fibers were transported by a high-speed air stream moving perpendicularly to a ribbon beam of light. The light blocked by an individual fiber was directly proportional to its mean optical diameter and time of flight in the sampling volume [5]. The blocked-light attenuation signal was analyzed by AFIS-L&D as length by weight and number and fiber diameter. The 40° angle light-scattering signal was analyzed by AFIS-F&M as fiber circularity (degree of fiber wall thickening, θ) and cross-sectional area by number $A(n)$. Fiber perimeter P was calculated from $A(n)$ and θ according to the formula, $\theta = 4\pi A(n)/P^2$, where the units of $A(n)$ and P are μm^2 and μm, respectively. Immature fiber fractions (IFF) were derived from the individual sample distributions of θ, where IFF was defined as the percent of fibers of $\theta < 0.25$ and $\theta = 1.00$ for a perfect (non-collapsed) circle. Fine fiber fractions (FFF) were similarly obtained from the distributions of $A(n)$; FFF was the percent of fibers of $A(n) < 60\ \mu m^2$.

The mean sample size in the fiber maturation rate study was 8579.4 ± 310.5 fibers (maximum AFIS fiber count from 56-DPA bolls = 10,000; minimum count from 21-DPA bolls = 507 fibers). The mean number of fibers analyzed per mote was 3814.5 ± 159.1, and the maximum and minimum mote-fiber analysis counts were 7253 and 1331 fibers, respectively. Fiber availability was not a limiting factor in the PD3 maturity mapping study, and all AFIS-F&M samples contained 10,000 fibers.

X-Ray Fluorescence Calcium Analyses

Fibers from all bolls of chronological age ≥ 21 DPA in the fiber maturation rate study (DES119, DPL5415, and Pima S-6) were analyzed first by AFIS with the fiber counter set at 10,000 fibers or (in the less mature bolls) a lower number consistent with the fiber weight available. The smallest sample size was 500 fibers (250 mg). The post-AFIS fiber samples were collected and calcium concentrations determined using x-ray fluorescence (Ca-XRF) analyses by a Kevex EDX-771 spectrometer (Fisons Instruments, San Carlos, CA). The analytical technique and Ca-XRF calibrations were described previously [13]. The 14-DPA DES119 and Pima S-6 fibers were analyzed by Ca-XRF only because fiber development in these least mature bolls was not sufficiently advanced for AFIS analysis.

STATISTICAL ANALYSES

All fiber maturation rate data from AFIS-F&M, *i.e.*, $A(n)$, θ, and micronAFIS, and Ca-XRF, were analyzed as completely random two-way (DPA × genotype) designs [10, 12]. Similarly obtained DPL51 mote data were analyzed as completely random designs (CRD, 3 mote locule positions × 3 mote weight classes). In a second set of CRD analyses, AFIS-F&M and Ca-XRF data from normal DPL51 seeds were included as a fourth "locule position" data set. The PD3 data for the same fiber quality parameters were also analyzed as completely random designs (node × position), and the mote data were a two-way CRD (weight × locule position). Maturity parameter means were separated, after one-way analyses of variance, by Tukey's least significant dif-

ference testing [12]. Fiber maturation rates for DES119, DPL5415, and Pima S-6 were determined by regression analyses of changes in $A(n)$, θ, micronAFIS, or calcium level over time (21 to 56 DPA). Correlations between $A(n)$, θ, or micronAFIS and Ca-XRF were obtained by regressing AFIS-F&M quantities on the corresponding Ca-XRF data.

Results

UPLAND AND PIMA COTTON MATURITY LEVELS AND MATURATION RATES

Genotype, DPA (chronological maturity), and the genotype × DPA interactions were all significant ($p = 0.001$) for the maturity estimates, micronAFIS, $A(n)$, θ, and Ca-XRF. Means of these maturity estimates for DES119, DPL5415, and Pima S-6 are shown in Table II. Minimal fiber development at 14 DPA prevented evaluation of the least mature (14 DPA) fibers by AFIS-F&M. At 56 DPA, genetic differences between Upland and Pima cotton varieties were apparent in all four maturity parameters. Pima S-6 fibers were appropriately lower in micronaire and cross-sectional area than fibers of the two Upland varieties, DES119 and DPL5415. Circularity was more sensitive to boll position and bloom date than were $A(n)$ and micronAFIS. The 56-DPA Pima S-6 and DPL5415 fibers from the August-bloom bolls were less mature, based on θ, than fibers from the July-bloom bolls of those varieties.

The Ca-XRF (calcium concentration by weight) data quantify dilution of the calcium-rich fiber primary cell wall by the predominantly cellulosic secondary cell wall and are thus estimates of fiber physiochemical maturity [13]. In this study, Ca-XRF data showed the same genotype and bloom-date patterns seen in θ, $A(n)$, and micronAFIS. When the three AFIS-F&M physical maturity parameters were regressed on the corresponding Ca-XRF data (Figure 1), DPL5415 and DES119 micronAFIS and θ were closely related to Ca-XRF (linear $r > 0.900$). The DPL5415 $A(n)$ was also correlated with Ca-XRF, regardless of bloom date (linear $r > 0.945$), and the correlation between DES119 $A(n)$ and Ca-XRF was linear but less close (linear $r > 0.753$). Pima S-6 relationships between AFIS-F&M physical maturity and Ca-XRF physiochemical maturity quantitations were also best fit by linear plots, but correlation coefficients varied from $r = 0.413$ for θ and $r = 0.531$ for micronAFIS from the 1993 Pima S-6 August bloom to $r = 0.822$ for θ (Pima S-6 in 1992). The poor correlations between micronAFIS or θ and Ca-XRF in the Pima S-6 August-bloom data resulted from minimal fiber-wall filling (Figures 1a,c) due to late-season suboptimal temperature conditions between mid-August flowering and mid-October harvest. The choice in Figure 1 of Ca-XRF axis ranges that matched the Pima S-6 Ca-XRF maximum (1925 ppm at 21 DPA) and minimum (1051 ppm at 56 DPA) resulted in "apparent" negative micronAFIS and θ estimates for DPL5415. Maximum

TABLE II. Ca-XRF and AFIS-F&M maturity estimates for DES 119, DPL 5415, and Pima S-6. All values shown are means of six determinations..

Genotype	DPA	Year/flowering date	Micron-AFIS	$A(n)$, μm^2	Theta	Ca-XRF, $mgkg^{-1}$
DES 119	14	1992	–	–	–	2730.0 ± 68.6
	21	1992	0.50 ± 0.10	89.33 ± 0.82	0.214 ± 0.01	2733.3 ± 36.6
	28	1992	1.37 ± 0.32	81.27 ± 2.62	0.290 ± 0.02	1733.3 ± 33.3
	35	1992	3.82 ± 0.41	118.82 ± 3.39	0.438 ± 0.03	1466.7 ± 36.7
	56	1992	5.11 ± 0.91	127.07 ± 16.54	0.565 ± 0.07	990.8 ± 18.1
DPL 5415	21	1993/July	1.01 ± 0.25	75.13 ± 3.42	0.269 ± 0.02	1483.0 ± 66.9
	35	1993/July	4.07 ± 0.25	120.20 ± 3.42	0.461 ± 0.02	1111.0 ± 78.8
	42	1993/July	6.74 ± 0.30	146.00 ± 4.19	0.651 ± 0.02	785.1 ± 81.8
	56	1993/July	6.08 ± 0.18	139.00 ± 2.53	0.605 ± 0.01	863.9 ± 49.4
DPL 5415	21	1993/Aug.	0.63 ± 0.30	81.41 ± 4.19	0.227 ± 0.02	1500.0 ± 81.9
	56	1993/Aug.	5.55 ± 0.25	135.6 ± 3.42	0.559 ± 0.02	810.9 ± 66.9
PIMA S-6	14	1992	–	–	–	2425.0 ± 32.0
	21	1992	0.33 ± 0.64	70.07 ± 7.25	0.214 ± 0.05	1925.0 ± 20.0
	56	1992	4.15 ± 0.67	96.69 ± 10.53	0.533 ± 0.05	1050.9 ± 56.7
PIMA S-6	21	1993/July	0.33 ± 0.27	57.80 ± 6.02	0.231 ± 0.02	1467.0 ± 94.7
	35	1993/July	2.88 ± 0.56	82.51 ± 6.18	0.442 ± 0.05	1167.0 ± 81.9
	42	1993/July	4.05 ± 0.58	92.91 ± 7.82	0.543 ± 0.04	1195.0 ± 85.9
	56	1993/July	4.25 ± 0.39	95.60 ± 6.73	0.559 ± 0.03	1101.0 ± 68.7
PIMA S-6	21	1993/Aug.	0.37 ± 0.01	70.76 ± 3.52	0.219 ± 0.00	1500.0 ± 94.9
	56	1993/Aug.	2.79 ± 0.73	81.32 ± 10.89	0.433 ± 0.01	1617.9 ± 50.0

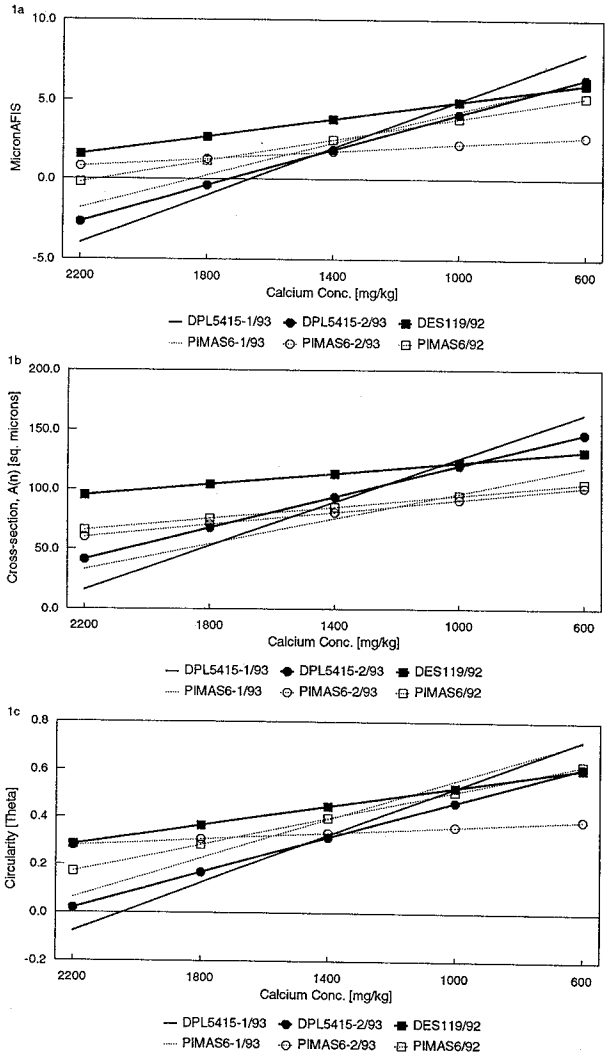

FIGURE 1. (a) Comparative rates of change in micronAFIS and relative calcium concentrations by weight, as determined by Ca-XRF. (b) Correlations between fiber cross-sectional area $A(n)$ and fiber cell-wall calcium levels during fiber maturation. (c) Correlations between fiber circularity θ and Ca-XRF over time. The cotton varieties were 1993 July-bloom DPL5415 (DPL5415-1/93), 1993 August-bloom DPL5415 (DPL5415-2/93), 1992 DES119 (DES119/92), 1993 July-bloom Pima S-6 (PimaS6-1/93), 1993 August-bloom Pima S-6 (PimaS6-2/93), and 1992 Pima S-6 (PimaS6/92). All plots were derived from linear regression equations based on AFIS and Ca-XRF data, and the Ca-XRF axis range was chosen to encompass Pima S-6 maximum and minimum calcium concentrations.

DPL5415 Ca-XRF values were 1500 ppm at 21 DPA and 78.5 ppm at 42 DPA.

The Ca-XRF values for mature fibers of Upland cotton varieties converged at <900 mgkg^{-1}, independent of variety [13]. Convergence points between 1000 and 900 mgkg^{-1} also occurred in the plots against Ca-XRF of DPL5415 and DES119 micronAFIS, $A(n)$, and θ (Figure 1). Plots of these AFIS-F&M maturity parameters against Ca-XRF for Pima S-6 showed convergence points in the same calcium concentration range, excepting the grossly immature 1993 August-bloom fibers (Figure 1a,c). The $A(n)$ plots for the genetically finer Pima S-6 converged at a 1000 to 900 mgkg^{-1} calcium concentrations, corresponding to a lower $A(n)$ than those of the Upland varieties (Figure 1b). This clear demonstration of the genetic differences between Upland and Pima fiber fineness suggests that increased accuracy of AFIS-F&M quantitations of Pima $A(n)$ and, probably, micronAFIS could be achieved through the use of separate Upland and Pima calibration standards.

We used the capacity of AFIS-F&M, combined with Ca-XRF corroboration, to quantify fiber maturity parameters in determining maturation rate equations for DPL5415, DES119, and Pima S-6 (Figure 2). Among the Upland varieties, physical maturation rates, quantified as micronAFIS, ranged from 0.12 to 0.14 micronAFIS units per day (Figure 2a). The most rapid micronAFIS increases occurred in the August-bloom fibers DPL5415 (0.14 units/day) and Pima S-6 (0.11 units/day). Correlation coefficients were >0.850, except for 1993 July-bloom Pima S-6 (linear $r = 0.753$).

Rates of primary wall dilution by secondary wall deposition, as quantified by Ca-XRF (Figure 2b), were highest in 1992 (DES119 and Pima S-6). In 1993, this primary : secondary ratio dilution rate was highest in July-bloom DPL5415. Very little secondary wall material was deposited in fibers of August-bloom Pima S-6. The Ca-XRF physiochemical maturation rate of August-bloom DPL5415 was the same as that of July-bloom Pima S-6.

Species differences in fiber cross section persisted throughout fiber maturation (Figure 2c). Upland fiber $A(n)$ increased at rates of 1.09 ± 0.19 (DES119), 1.48 ± 0.21 (July-bloom DPL5415), and 1.55 ± 0.26 (August-bloom DPL5415) μm^2/day. Rates of Pima S-6 $A(n)$ increases ranged from 0.30 ± 0.45 μm^2/day for August-bloom fibers in 1993 to 0.76 ± 0.13 μm^2/day in 1992 and 0.93 ± 0.13 μm^2/day in 1993 (July-bloom). All $A(n)$ accumulation rates were linear ($r > 0.800$) except the fiber thickening rate of the stressed August-bloom Pima S-6. Characteristic Pima fiber fineness was apparent in the consistently lower 56-DPA $A(n)$ values (Figure 2c).

Cell-wall thickening, estimated as AFIS-F&M θ (Figure 2d), indicated no significant varietal differences in fiber physical maturation rates, which were all linear ($0.805 < r < 0.957$). Species differences were apparent in the relative degrees of cell-wall thickening of both July- and August-bloom DPL5415 and Pima S-6. In 1992, no species differences in relative fiber circularity were

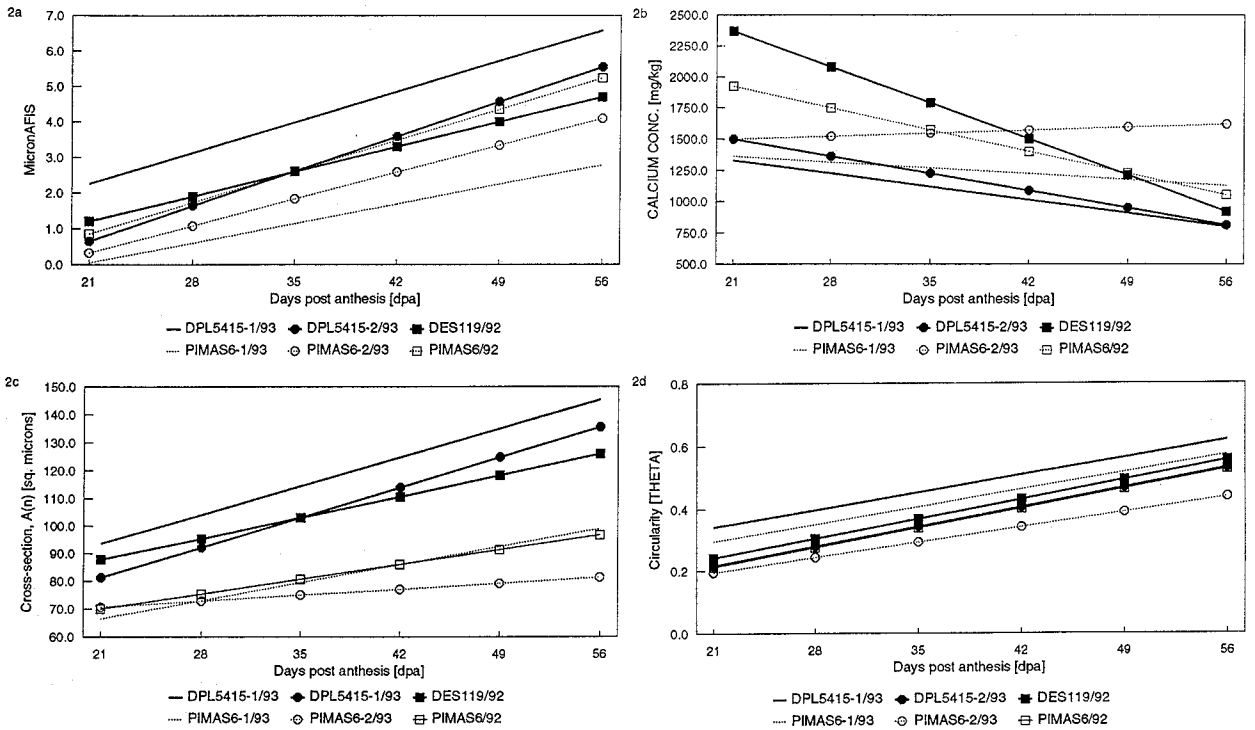

FIGURE 2. (a) Rates of micronAFIS increase over time. (b) Rates of decrease over time in fiber cell-wall calcium concentration (Ca-XRF) by weight. (c) Rates of fiber cross-sectional area $A(n)$ increase over time. (d) Rates of fiber cell-wall thickness, circularity, increase over time. The cotton varieties were the same as those in Figure 1. All plots were derived from linear regression equations based on AFIS and Ca-XRF data.

apparent between Upland DES119 and Pima S-6. The immature fiber fractions (IFF) and fine fiber fractions (FFF) derived from the DES119, DPL5415, and Pima S-6 circularity, θ, and $A(n)$ distributions, respectively, are shown in Table III.

MATURITY LEVELS OF LONG-FIBERED MOTES

The degree of cotton fiber secondary wall thickening determines most of the technological features of the fiber [15]. Fiber cell-wall thickness, estimated by AFIS-

TABLE III. Immature fiber fractions (IFF) and fine fiber fractions (FFF) of DES119, DPL5415, and Pima S-6. All values shown are means of six determinations. Means associated with the same variety and followed by the same lower case letter are not significantly different ($P = 0.01$).

Genotype	DPA	Year/flowering date	IFF, %		FFF, %	
DES 119	21	1992	67.5 ± 2.6	a	18.6 ± 0.3	b
	28	1992	40.3 ± 6.1	a	27.6 ± 3.6	a
	35	1992	14.2 ± 3.4	b	6.3 ± 1.2	c
	56	1992	7.7 ± 3.9	c	9.6 ± 4.5	c
DPL 5415	21	1993/July	48.1 ± 1.5	b	36.7 ± 1.1	a
	35	1993/July	13.5 ± 1.5	c	5.1 ± 1.1	c
	42	1993/July	5.9 ± 1.9	c	5.5 ± 1.3	c
	56	1993/July	6.3 ± 1.1	c	7.4 ± 0.8	c
DPL 5415	21	1993/Aug.	59.4 ± 1.9	a	28.1 ± 1.3	b
	56	1993/Aug.	8.1 ± 1.5	c	7.5 ± 1.1	c
PIMA S-6	21	1992	64.3 ± 6.1	a	45.7 ± 9.3	ab
	56	1992	12.1 ± 3.2	b	23.9 ± 8.1	b
PIMA S-6	21	1993/July	57.15 ± 6.3	a	60.6 ± 7.7	a
	35	1993/July	18.1 ± 5.6	b	31.2 ± 7.8	b
	42	1993/July	11.0 ± 2.7	b	25.5 ± 6.9	b
	56	1993/July	11.5 ± 3.2	b	26.5 ± 5.8	b
PIMA S-6	21	1993/Aug.	62.3 ± 16.4	a	46.2 ± 8.7	ab
	56	1993/Aug.	11.3 ± 3.2	b	36.7 ± 4.2	b

F&M $A(n)$ and circularity, is directly related to fiber maturity and is a factor in both fiber micronaire and physical fineness. Boll-level relationships between fiber chronological maturity and fiber physical and physiochemical maturity have been described above. The major component of cotton fiber variation is the individual seed [2], however, and significant variations in both seed weight and fiber maturity are found within individual bolls and locules [3, 4, 6, 7]. When we used AFIS-F&M and Ca-XRF sequentially to quantify fiber maturity associated with DPL51 long-fibered motes, *i.e.*, subnormal weight seed structures bearing normal (24.6 ± 0.8 mm) length fibers, mote micronAFIS, cell-wall calcium concentration, and fiber circularity were all significantly affected by mote weight (Figure 3a,b,d). The position of the motes within the locule (apical, medial, or basal) also modified relative calcium content and fiber circularity. Fiber cross section (Figure 3c) was unaffected by either mote weight or mote position in the locule, and there were no significant interactions between mote weight and mote position. Minimum micronAFIS, $A(n)$, and θ were consistently associated with the lowest weight motes from the apical locule position. The Ca-XRF physiochemical maturity assay also indicated that the least mature fibers occurred at that combination of mote-weight and locule position. Mote IFF values ranged from 41.4 ± 3.2% for the lowest weight apical motes to 20.4 ± 0.9% for the heaviest medial motes.

MicronAFIS, Ca-XRF, $A(n)$, and θ means for normal-seed DPL51 fiber were included in Figure 3 to simplify mote to seed comparisons. Average mote micronAFIS was 2.40 ± 0.47, compared to normal-seed micronAFIS of 6.06 ± 0.44. Thus, mote micronAFIS was analogous to micronaire in the low discount range when the normal-seed DPL51 micronaire was in the high discount range. (Mote mean $A(n)$ was 84.6 ± 4.9 μm^2, compared to the normal-seed mean of 136.1 ± 6.1 μm^2.) These $A(n)$ means corresponded to a mote FFF of 33.6 ± 6.3% and normal-seed FFF of 20.1 ± 10.6. Circularity, the cell-wall thickening component of fiber physical maturity, was more sensitive than $A(n)$ to mote weight and locule position. Mean mote θ and IFF were 0.377 ± 0.038 and 27.8 ± 7.1%; normal seed θ and IFF were 0.611 ± 0.01 and 4.8 ± 1.5%. The DPL51 mote Ca-XRF mean was 1611.1 ± 336.5 $mgkg^{-1}$ calcium. The corresponding normal-seed mean was 779.2 ± 48.2 $mgkg^{-1}$.

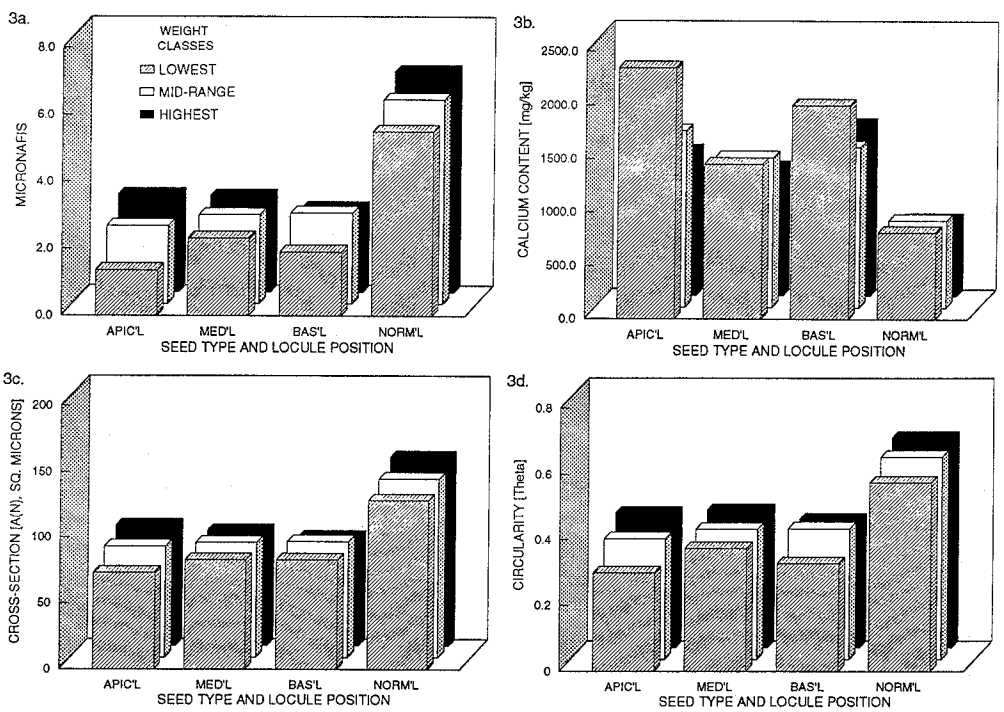

FIGURE 3. (a) MicronAFIS values of DPL51 seeds and long-fibered motes. Motes fell into three weight classes (7–20, 21–35, or 36–59 mg) and were selected from apical, medial, or basal locule positions. (b) Comparison of cell-wall calcium levels of DPL51 seeds and motes. (c) Mote and seed cross-sectional areas $A(n)$ according to weight and locule position. (d) Mote and seed fiber circularities θ by weight and locule position.

MOTE EFFECTS ON COMPOSITE FIBER MATURITY AND UNIFORMITY

The DPL51 normal-seed and mote fiber-maturity estimates discussed above were the means of AFIS-F&M and Ca-XRF quantitations for individual seeds or motes. Bale (HVI) micronaire is a composite of seed- and mote-fiber micronaire values and depends on the relative numbers of motes and normal seeds present on the plants from which the bale of fiber was harvested. The number of long-fibered motes present is particularly important to composite fiber maturity, since the fibers on such motes are of normal length and, consequently, are not removed during ginning [8].

Both fiber maturation rates and the number of long-fibered motes per locule or boll, *i.e.,* underweight seeds bearing normal-length fibers, are strongly and negatively influenced by suboptimal environmental conditions, particularly temperature, rainfall, and insolation [3, 4]. These three environmental factors interacted to increase the number of motes found at most fruiting sites on *irrigated* PD3 plants grown in South Carolina in 1992 (Figures 4a,b). We considered only positions 1 and 2 on nodes 7 through 18, since central, main crop fruiting sites produced more than 86% of the yield. Early season drought limited boll load on the lower nodes of the rainfed plants (Figure 4a). Rain and extended periods of cloudy weather [3] decreased the amount of photosynthate available for partitioning among bolls during fiber development, and this metabolic resource limitation was exacerbated in the irrigated plants by increased competition among the more numerous bolls retained during the early season drought. Irrigated PD3 bolls contained elevated numbers of low-weight seeds bearing more immature fibers (Figures 4b,d). The composite AFIS-F&M micronAFIS mean for all rainfed (Figure 4c) PD3 fruiting sites was 4.4 ± 1.3, and the irrigated (Figure 4d) composite micronAFIS mean was 3.5 ± 1.2. Comparable means of four commercial micronaire determinations for the 1992 PD3 crop were 4.2 ± 0.2 (rainfed) and 3.8 ± 0.1 (irrigated).

Both the cross-sectional and circularity components of micronAFIS were affected by those environmental factors that increased mote counts and decreased fiber maturity (Figure 5). Fibers from irrigated PD3 plants were finer and less mature than those from rainfed plants. The composite rainfed $A(n)$ mean equivalent to the distribution shown in Figure 5a was 108.6 ± 17.2 μm^2; the corresponding irrigated PD3 $A(n)$ mean (Figure 5b) was 100.2 ± 19.2 μm^2. These $A(n)$ means corresponded to fine fiber fractions of 17.9 ± 11.5% (rainfed) and 20.6 ± 12.3% (irrigated). The rainfed FFF mean incorporated a maximum locule FFF of 72.8% from node 15, position 1, and a minimum locule FFF of 3.2% at node 10, position 1. The irrigated FFF mean contained a maximum locule FFF of 66.5% at node 18,

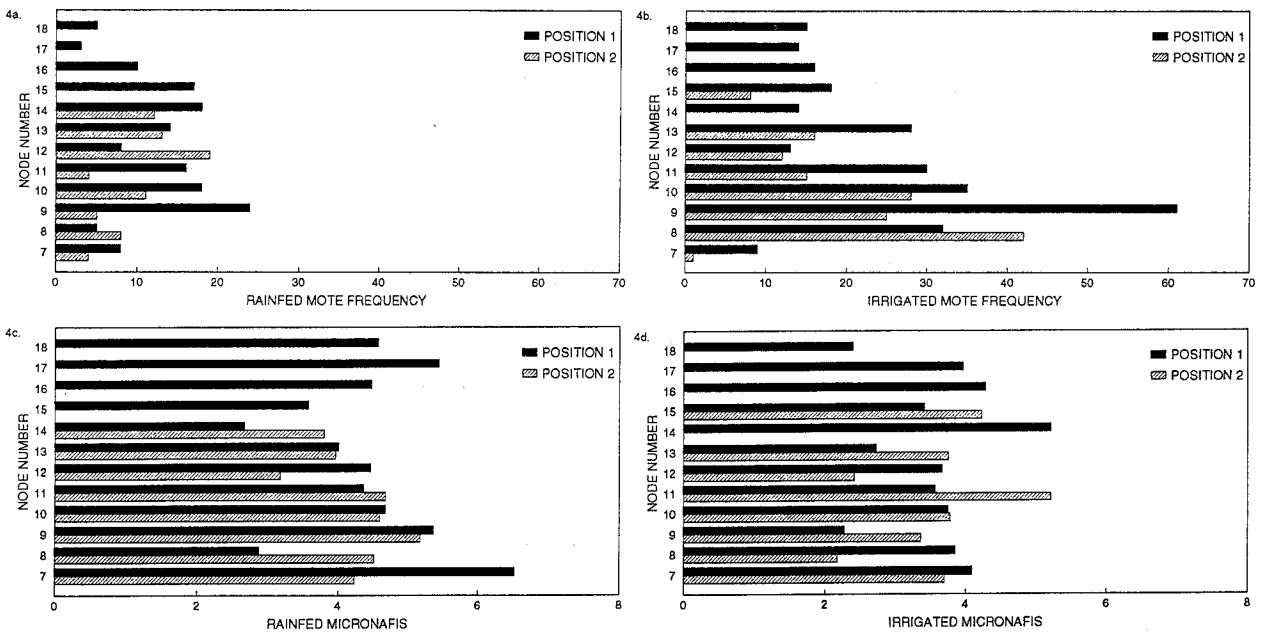

FIGURE 4. (a) PD3 mote frequency, by fruiting site, under rainfed conditions. (b) PD3 mote frequency under irrigation. (c) MicronAFIS distribution by fruiting site under rainfed conditions. (d) MicronAFIS distribution by fruiting site under irrigation.

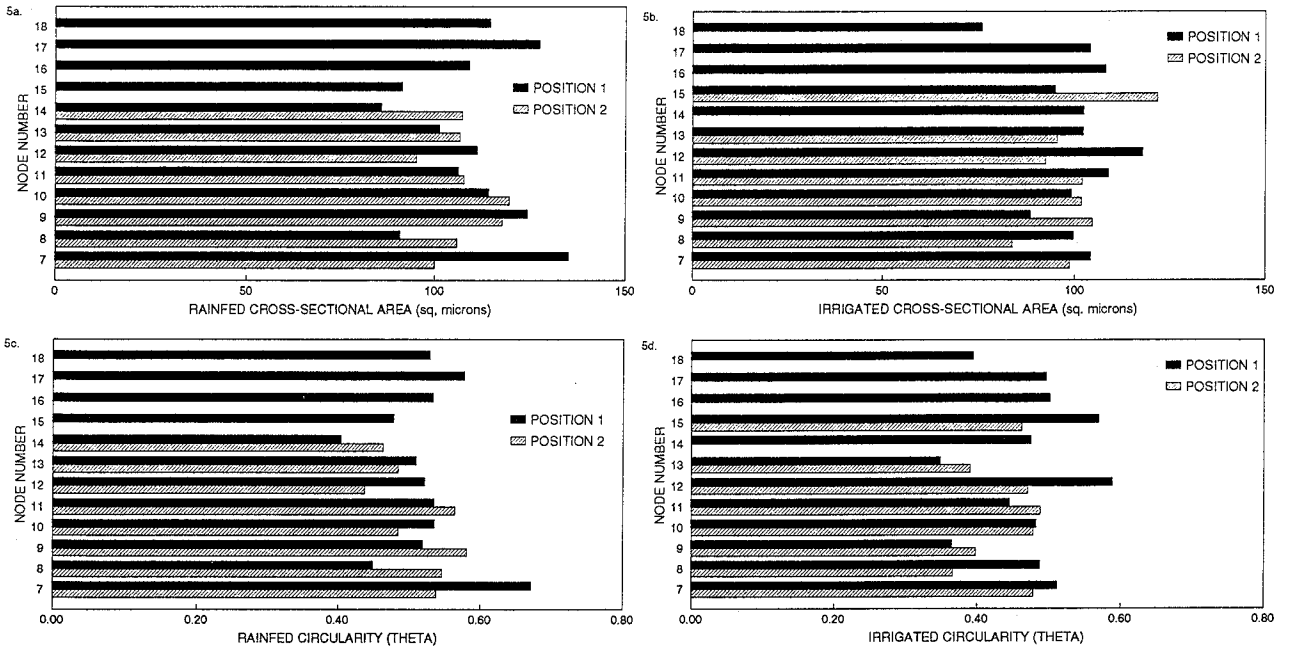

FIGURE 5. (a) Distribution by fruiting site of cross-sectional areas $A(n)$ of rainfed PD3 fiber, (b) $A(n)$ distribution by fruiting site of irrigated PD3 fiber, (c) distribution of fiber circularities θ by fruiting site of rainfed PD3 fibers, (d) distribution of fiber circularities θ by fruiting site of irrigated PD3 fibers.

position 1, and a minimum locule FFF of 2.7% at node 15, position 1.

Circularity was more clearly influenced by environment and metabolic resource competition (Figures 5c,d). Rainfed composite θ mean was 0.521 ± 0.089; irrigated composite θ mean was 0.462 ± 0.092. These circularities corresponded to a rainfed immature fiber fraction of $12.9 \pm 9.2\%$ and an irrigated IFF of $16.7 \pm 10.5\%$. The composite rainfed IFF mean included a locule maximum of 75.0% at node 14, position 1, and a locule minimum of 2.5% at node 10, position 2. Within the irrigated composite IFF mean, the maximum locule IFF was 63.1% at node 8, position 2, and the minimum locule IFF was 3.5% at node 15, position 1. The wide ranges of fiber maturities found at fruiting sites within the main crop suggest that the bale-level HVI maturity estimates do not adequately represent either the degree or uniformity of crop fiber maturity. Optimizing bulk (bale) fiber maturity and uniformity through either breeding or improved production practices will require examination and mapping of "point-source" fiber variations at the boll and locule level, and the sort of small-sample fiber quality analysis best done by AFIS-F&M.

Conclusions

The power and potential of AFIS-F&M as a tool in cotton fiber maturity research are only now being realized and used. AFIS-F&M, particularly when combined with Ca-XRF, permits quantitation of fiber maturity parameters at the boll, locule, and individual seed levels. Using the small-sample analytical capabilities of AFIS-F&M, micronaire, the standard estimate of fiber maturity, can be partitioned into fineness (cross section) and maturity (circularity) components, thus allowing a factor by factor examination of the effects of growth environment and genetics on both the degree and uniformity of fiber maturity. The AFIS-F&M will also prove invaluable in developing predictive fiber maturity models that integrate whole-plant growth models, *e.g.*, GOSSYM-COMAX, and fiber-quality based processing models like the engineered fiber system (EFS®).

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