Quantitative Cotton Fiber Maturity Measurements by X-ray Fluorescence Spectroscopy and Advanced Fiber Information System

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Cotton fiber maturity is an important factor in cotton classification and fiber and textile processing. The objective of this study was to test the hypothesis that during fiber maturation, structural calcium in the primary wall is diluted by the development of the highly cellulosic secondary wall. Increases in this dilution effect on relative calcium concentration were found to parallel increasing chronological maturity in fibers harvested from 20 to 56 days after flowering. Fiber samples were evaluated with the Advanced Fiber Information System, which reported fiber quality parameters related to and including the cross-sectional area and circularity. Calcium concentrations were obtained using X-ray fluorescence spectroscopy. There were significant inverse relationships between both fiber circularity and cross-sectional area measurements with relative calcium concentration. These relationships were independent of cotton variety or growing conditions. Calcium determination by X-ray fluorescence offers a simple method for determining cotton fiber maturity.

Keywords: *Cotton; fiber maturity; calcium; X-ray fluorescence; AFIS*

INTRODUCTION

Cotton fiber maturity has a direct relationship to fiber quality. Immature fibers adversely affect the spinnability and dyeability of the yarn (Smith, 1991; Hughs et al., 1988; Deussen, 1992). Maturity of cotton fiber is directly related to the accumulation of the highly cellulosic secondary wall that begins to thicken after 20 days postanthesis (DPA) (Mauney and Stewart, 1986). An individual immature cotton fiber is a single hyperelongated plant cell bounded by a primary cell wall consisting of a matrix of cellulose and hemicelluloses held together by calcium-rich pectins (DeLanghe, 1986; Goldberg, 1985). During formation of the secondary wall, relative concentrations of noncellulosic structural constituents such as calcium decrease as the proportion of cellulose increases dramatically (Delanghe, 1986; Leffler and Tubertini, 1976). This dilution effect is particularly apparent in cell constituents characteristic of the primary wall such as calcium. The secondary wall is approximately 99% cellulose, and the amount of secondary wall present is directly related to fiber maturity measured as degree of wall thickening (Lord and Heap, 1988). Immature fibers have thinner cell walls which ultimately affect spinnability and yarn quality (Deussen, 1992). The amount of dye that diffuses into the pores of the fiber is related to the amount of cellulose in the secondary wall (Watson and Jones, 1985).

Cotton classification has historically relied on the physical characteristics of fibers. While there are many maturity tests in existence, few are quantitative and relate directly to dyeability (Smith, 1991). The physical attributes of the fiber are informative, but the biochemical characteristics can be important as well. In tandem, biochemical and physical maturity tests can provide fiber maturity evaluations that are beneficial to the grower and the processor. The Advanced Fiber Information System (AFIS) and X-ray fluorescence spectroscopy (XRF) are two complementary tests that can be quantitative indicators of maturity.

The Zellweger–Uster Advanced Fiber Information System (AFIS) was developed to rapidly measure essential cotton fiber property distributions such as length, diameter, maturity, and fineness (Bragg and Shofner, 1993). The system individualizes and cleans the fibers before presentation to an electro-optical sensor. High-velocity air flow moves individualized fibers past the optical sensor. The fibers present in the samples generate characteristic electrical signals. The interruption by the moving fibers of the light beam impinging on the electro-optical sensor produces two types of signals of interest. One signal results from the light beam being blocked by the fiber in proportion to its mean optical diameter and in direct relation to its time of flight in the sampling volume. The other is the result of the light scattered by the same fiber at 40° from the beam direction. Data from the attenuated signal are used to directly measure individual fiber length and diameter. Data from the 40° scattering signal yield fineness and maturity measurements.

X-ray fluorescence is an analytical technique that uses 1–100 keV electromagnetic radiation for sample excitation. Emitted X-rays are uniquely characteristic of the element present. An energy dispersive X-ray fluorescence spectrometer can generally detect elements with atomic numbers of 11 and higher depending upon the detector utilized. The monoenergetic nature of X-rays from secondary targets serves to improve peak to

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background ratios as well as minimum detection limits by customizing the analysis to a select group of elements (Bertin, 1970; *Kevex XRF Spectrometer 770 User's Manual*, 1989). The method can be used to analyze cotton fiber samples as small as 100 mg for many elements. The main advantages of X-ray fluorescence are speed of analysis, minimal sample preparation, and small sample size. It can follow the relative changes in concentration of many elements contained in cotton fiber during maturation.

In this study, we compared XRF with AFIS analyses of fiber of known chronological maturity to determine the applicability of the chemical test for use as a quantitative measure of fiber maturity. By analyzing fibers with both methods, we also examined the coordinated changes in physical and chemical properties of cotton during maturation.

MATERIALS AND METHODS

Boll Harvesting and Fiber Preparation. *Field-Grown Cotton Varieties.* DPL5415 and DES 119 (*Gossypium hirsutum*) and Pima S-6 (*G. barbadense*) cotton was field-grown at Mississippi State, MS, in 1992 and 1993. Flowers were tagged on the day of anthesis, and bolls were harvested at 21, 28, and 56 days postanthesis (DPA). Bracts and stems were removed from the bolls and fresh weights recorded. Bolls were cut open carefully to avoid contamination of the lint with burr and frozen thoroughly. Bolls were freeze-dried for more than 48 h to remove water and then separated into burr, lint, and seed. The seed coat of immature seeds did not adhere to the seed and was separated with the lint. The individual boll components were weighed and stored frozen for further analyses reported elsewhere. The lint from remaining bolls was placed in small envelopes and sent to the Southern Regional Research Center (SRRC) for fiber geometry and elemental analyses.

Greenhouse-Grown Cotton Variety. Deltapine 50 (DPL50) (*G. hirsutum*) plants were grown in the SRRC greenhouse during 1993. Flowers were tagged on the day of anthesis and collected 20, 31, or 45 DPA. Bolls were allowed to air-dry in the greenhouse before the fiber was separated from the seeds by hand. Seed counts were recorded and fiber qualities were determined at SRRC.

Instrumentation. Physical fiber parameters were calculated by the Advanced Fiber Information System by Zellweger–Uster, Inc., Knoxville, TN, equipped with a fineness and maturity module. Sample preparation consisted of drawing fibers by hand into slivers of suitable lengths as calculated by the AFIS program for the weight of fiber to be used for each analysis. The number of fibers per replication could be set as high as 10 000 or a lower number consistent with the amount of fiber available. The smallest fiber sample size used was 250 mg. If the present fiber count was reached, the excess unprocessed sample was backed out of the feed port. AFIS is calibrated using ASTM calibration cottons.

X-ray fluorescence analysis (XRF) was accomplished with a Kevex EDX-771 spectrometer from Fisons Instruments, Inc., San Carlos, CA. Exciting radiation was obtained with a rhodium continuum, and a titanium secondary target at 20 kV, 1 mA in a helium atmosphere. Spectra were acquired for 100 s. The detector is lithium drifted silicon. Samples were ground to pass a 20-mesh screen with a Wiley mill and pressed into a 31-mm-diameter pellet at 20 000 psi for 20 s with a hydraulic press. As a result, sample sizes as low as 100 mg could be and were analyzed. Standards were created with the NIST SRM 1515 Appleleaves by successive dilution with Whatman CC41 cellulose powder. Concentrations were calculated with the fundamental parameters method which accounts for absorption/enhancement effects between elements (Bertin, 1970). The minimum detection limit was 6 ppm, with a standard error for the calibration standards of 34.63 ppm. Concentrations were checked by sending a representative sample set to a private laboratory for inductively coupled plasma emission analysis (ICP). The 10% difference between ICP and XRF calculated concentrations was within acceptable quality assurance limits.

Table 1. Analysis of Variance for All Varieties with Theta (Circularity), Area (Cross-Sectional Area), and Calcium Concentration

		mean SQ[a]		
source	DF	theta	area	calcium
variety	3	0.09***	5933.13***	1663491.76***
age	2	0.55***	14653.22***	6384412.25***
var × age	6	0.02***	715.12***	679323.65***
error	60	0.002	92.07	30571.01
total	71			

[a] *, **, ***, significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2. Analysis of Variance for Immature Fiber Fraction (IFF), Fine Fiber Fraction (FFF), and Calcium Concentration

		mean sq[a]		
source	DF	IFF	FFF	calcium
variety	3	2206.24***	2461.64***	1663491.76***
age	2	10219.62***	3663.67***	6384412.25***
var × age	6	685.89***	356.33**	679323.65***
error	55	33.91	102.1	30571
total	71			

[a] *, **, ***, significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Statistical Analyses. The AFIS cross-sectional area, the AFIS circularity/fineness data, and the X-ray fluorescence data were analyzed as one-way analyses of variance with chronological age as the treatment (Sokal and Rohlf, 1981). Data for each fiber quality parameter were analyzed separately for each cotton variety or cropping year. The effects of variety and chronological age (DPA) on cross-sectional area, theta (circularity), and calcium were examined as completely randomized two-way factorial designs (3 DPA × 4 varieties). Analyses were made using MSTATC (1991).

RESULTS AND CONCLUSION

The chemical maturity marker, calcium, measured by X-ray fluorescence mirrors the physical fiber properties measured by AFIS. Statistical analyses show a strong correlation between calcium concentration and the shape parameters calculated by AFIS. The analyses of variance in Table 1 show not only significant differences over the growth period but also varietal differences. The equation for the circularity measurement as calculated by AFIS is

$$\Theta = 4\Pi A/P^2 \quad (1)$$

where A is the cross-sectional area in square micrometers and P is the perimeter in micrometers. The fine fiber fraction was defined as the percentage of fibers with cross-sectional area less than 60 μm^2 and the immature fiber fraction was the percentage of fibers with values of circularity less than 0.25Θ (Lord and Heap, 1988). According to statistical analyses, there were significant varietal and age differences contributing to changes in immature fiber fraction (IFF) and fine fiber fraction (FFF).

The analyses of variance for IFF and FFF are presented in Table 2. Mean values for calcium for each variety at each collection date showed trends when compared to theta and area in Figures 1–3. Calcium concentration changes over time were linear for field-

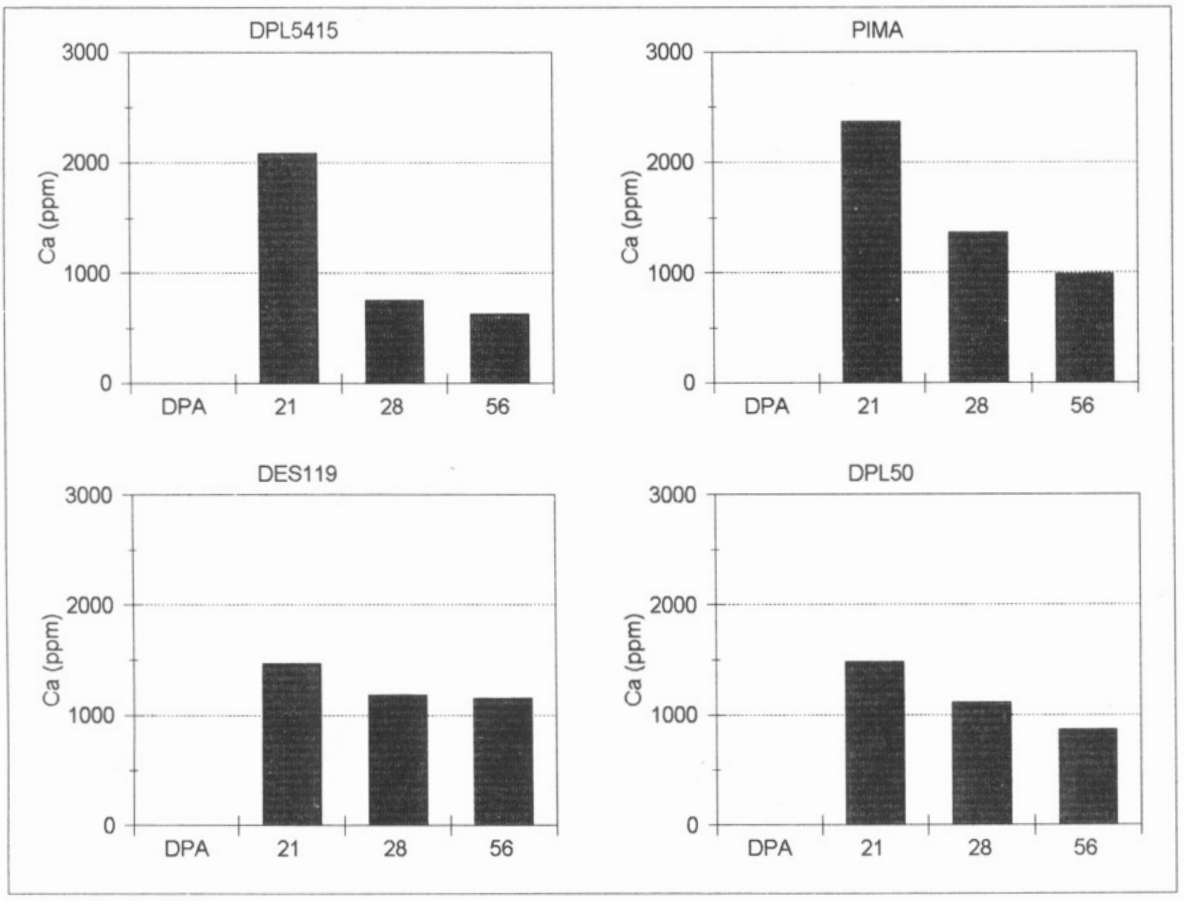

Figure 1. Means of fiber calcium concentration plotted against day postanthesis (DPA) for all varieties tested (DPL5415, PIMA, DES119, and DP50).

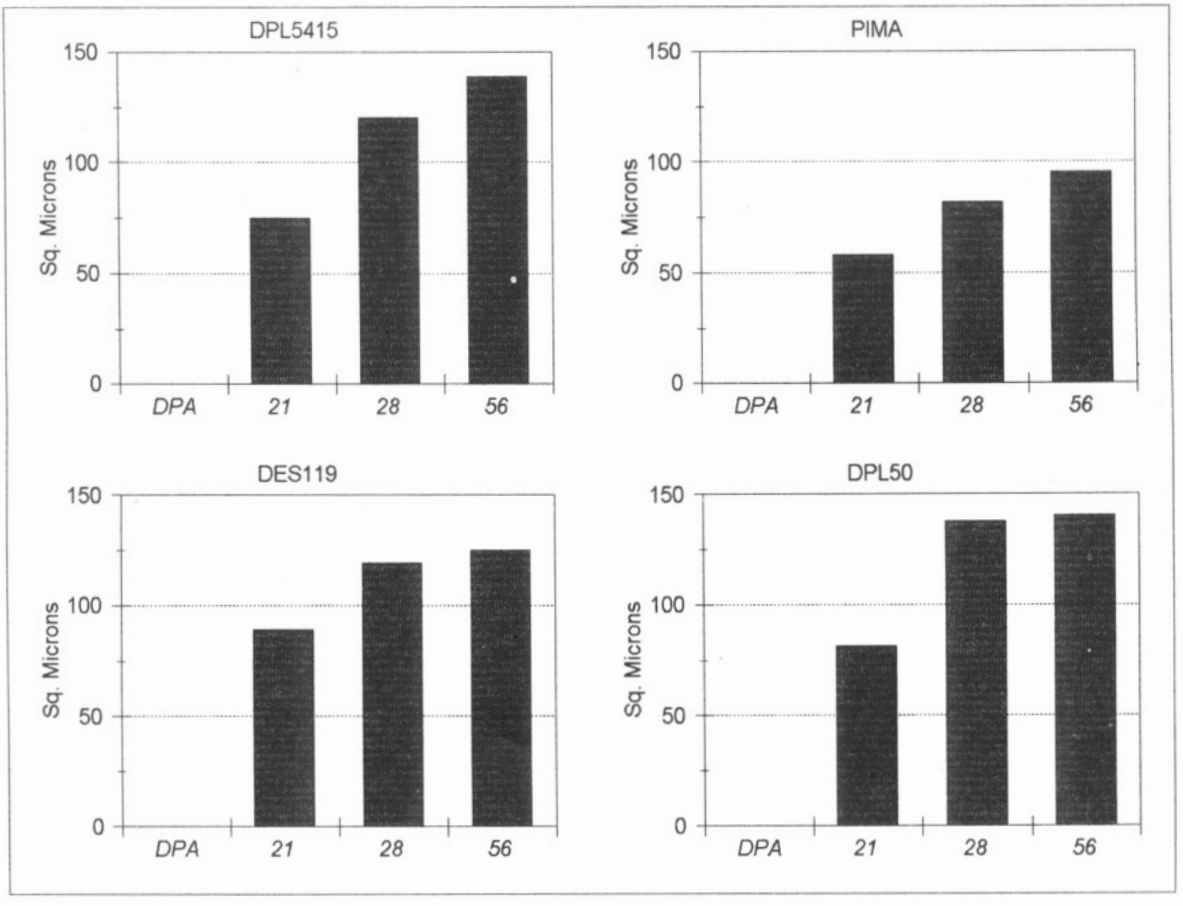

Figure 2. Means of area plotted against day postanthesis (DPA) for all varieties tested (DPL5415, PIMA, DES119, and DP50).

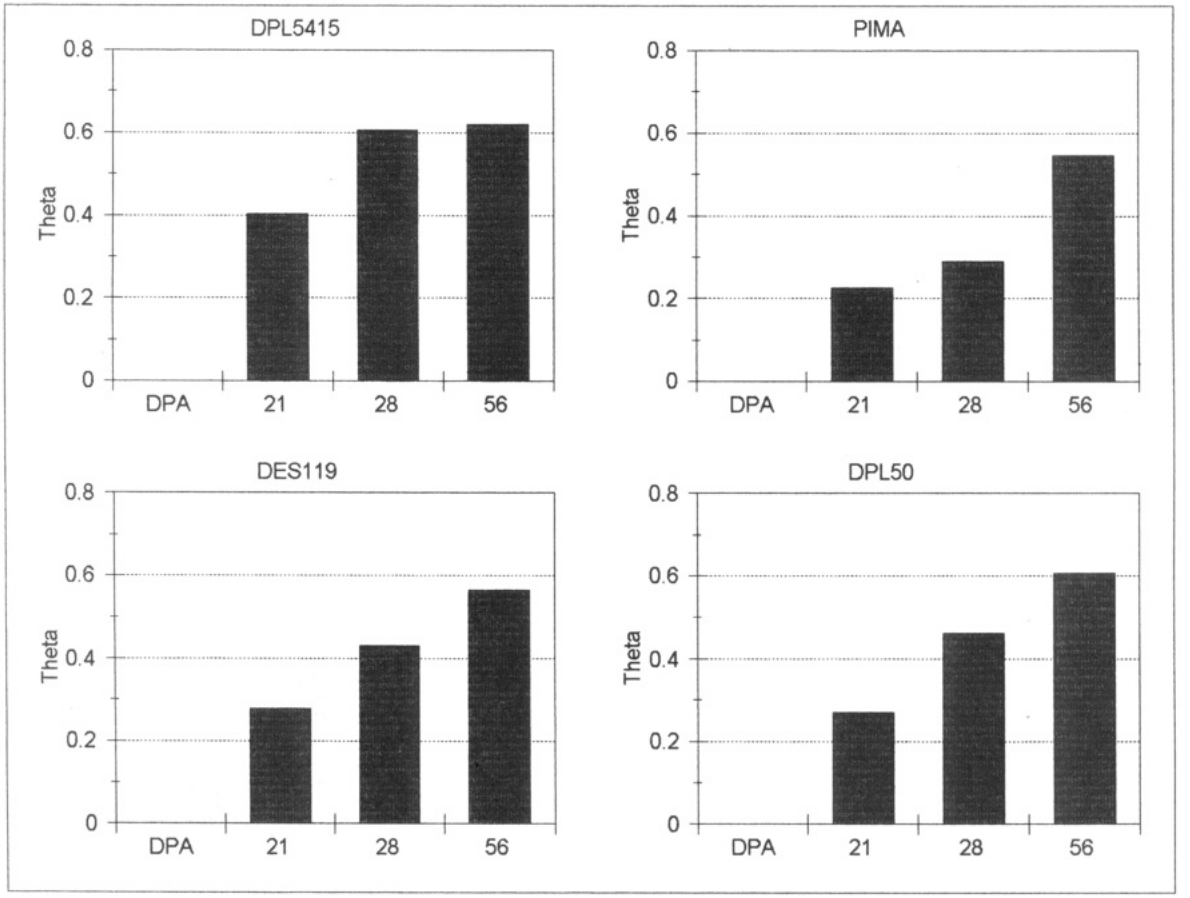

Figure 3. Means of circularity (theta) plotted against day postanthesis (DPA) for all varieties tested (DPL5415, PIMA, DES119, and DP50).

Table 3. Linear Regression Analysis of Day Postanthesis (DPA) vs Calcium Concentration for All Varieties

variety	dF	*r*	area incpt	slope
DPL5415	25	0.815	62.73	1.48
PIMA	25	0.819	46.99	0.93
DES119	18	0.8	64.92	1.09
DPL50	28	0.811	47.12	2.27
variety	dF	*r*	theta incpt	slope
DPL5415	25	0.858	0.173	0.008
PIMA	25	0.875	0.127	0.008
DES119	18	0.931	0.053	0.009
DPL50	28	0.814	0.275	0.008
variety	dF	*r*	calcium incpt	slope
DPL5415	25	−0.807	1648.8	−15.13
PIMA	25	−0.451	1506.9	−6.78
DES119	18	−0.92	2961.7	−35.81
DPL50	28	−0.741	2940.8	−55.86

grown DES119 and DPL5415 and curvilinear for greenhouse-grown DPL50 (Figure 1; Table 3). The regression lines for calcium showed significant differences between varieties in the slopes of the lines and the intercepts at the 99.99% confidence level (Table 3). However, there were no significant differences in the slopes for theta detected between varieties. These data also demonstrate that an inverse relationship exists between calcium concentration and the AFIS maturity calculations.

Pima *(G. barbadense)* fibers at maturity are characteristically longer and finer than fibers of *G. hirsutum* varieties. This genetic difference was apparent in all five maturity parameters in Tables 1 and 2. The Pima fiber cross-sectional areas and fiber calcium concentrations were significantly different from the varieties tested (Tables 1 and 3; Figures 1−3). Pima is a long-season, southwestern cotton not normally grown in Mississippi. The effect of the suboptimum growing conditions for Pima was shown in the *r* values in Table 3 as well as in Figures 1−3.

Calcium was chosen as a maturity marker because of its relative stability and localization in the primary wall and its easy detectability by X-ray fluorescence. AFIS parameters provide a basis of comparison because of the many measurements of fiber dimension that it provides. X-ray fluorescence requires little sample preparation, a minimum sample size of 100 mg, and analysis time of 100 s per sample, it is a rapid method for cotton classification or as a basis for comparison to current classification methods. Together, AFIS and XRF provide a more direct, quantitative measure of cotton fiber maturity than methods currently in use (Smith, 1991).

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