STATUS OF THE DEVELOPMENT OF CALIBRATION COTTONS FOR FIBER MATURITY

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Abstract

Results are reported on the continuing effort to produce large quantities of diverse cottons with well-defined values of fineness and maturity. We are using the reference method for measuring fiber maturity based microscopic image analysis of thin sections of fiber bundles. Fiber samples representing the range of properties of U.S. cottons were obtained from cooperating cotton breeders. Two different software packages for assessing fiber maturity by image analysis, one from Leica International and another from Professor Bugao Xu, University of Texas, are being evaluated. Findings of the research indicate that: a) the image analysis reference method gives reliable data with a minimum of problems and is at a point where the technology can be transferred to other laboratories; b) producing a range of fiber maturities by selective harvesting from the plant gives reasonable differences in fiber area, perimeter, and micronaire, but only marginal differences in maturity; c) results from Leica and Xu are compatible but Xu's software is faster and easier to use; and d) micronaire measurements alone are not good predictors of fiber maturity.

Introduction

An accurate measure of the maturity of a sample of cotton is essential for assessing the quality of the fiber. The reference method for measuring fiber maturity based microscopic image analysis of thin sections of fiber bundles was used to determine the area and perimeters of the individual fiber sections (Thibodeaux and Evans 1996). Fiber samples representing the range of properties of U.S. cottons were obtained from cooperating cotton breeders. Procedures for analysis included preparing a bundle of parallel fibers randomly selected from each fiber type, embedding the bundle in a metharcrylate matrix, sectioning the bundle with a microtome, and microscopic image analysis to determine the maturity each fiber in the thin sections (Boylston et al., 1995).

As originally outlined, a project has been initiated to develop a comprehensive set of cottons having a wide range of fiber maturities and finenesses that have been calibrated using the image analysis reference method (Thibodeaux 1998). The approach being followed to accomplish this was to assist at least two cooperating laboratories (the International Textile Center and the Bremen Fiber Institute) to develop their capabilities to prepare, mount, embed, and section representative bundles of cotton fiber and perform image analysis on thin fiber sections to measure the fiber area, perimeter, and circularity of representative fiber samples.

The objectives that are reported here include:

1. Continue screening samples obtained from our cooperators: Drs. John Pellow and H.B. Cooper, Boswell Corp., Bakersfield, CA; Dr. Richard Percy, USDA-ARS, Maricopa, AZ; Dr. John Gannaway, Texas A&M, Lubbock, TX; and Dr. Bill Meredith, Stoneville, MS. and attempt to make a decision as to which will be the final set of varieties with the range of genetic fineness and suitable yield to become our maturity calibration cottons.
2. Complete work on validating the image analysis software of Dr. Bugao Xu (Xu 1999) and coordinate the adoption and use by all three collaborators (SRRC, ITC, and Bremen) and continue to assure consistency in measurements between the three laboratories.
3. Improve the accuracy of the method by analyzing some selected cross-sections with high magnification (100 x oil-immersion) objective in hopes that the number of pixels in the fiber perimeter will be much smaller than the pixels in the cell wall. It is hoped that this would in effect reduce the signal to noise ratio uncertainty. Improve the precision of the method by checking the statistical significance of the number of samples and the number or reps to obtain confidence at the 99% and 95% degrees of confidence.

Results and Discussion

Continue Screening Samples Obtained From Cooperators

Results obtained with the subset of the twelve cultivars planted and hand harvested by the collaborators are shown in Table I. The cultivar name, the ranges of the average values of fiber wall area, perimeter, and degree of thickening (θ) as determined from image analysis have been included. This range of values covers data obtained with the samples harvested from the three growing zones on the plant. Also included are the range of Micronaire values measured on the same samples. For purposes of reference, results obtained in similar fashion with the ITMF reference cottons have been included. Clearly, the present samples cover as wide or wider of a range of fineness/maturity values as did the ITMF cottons. Although twelve cultivars are represented here, the table represents results obtained with three times that many

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cotton samples. Data obtained with these cultivars are ranked in the order of increasing Micronaire. For all of the samples Micronaire ranges from 2.6 to 6.2, wall area from 74 to 162 mm^2, perimeter from 45 to 60 µm, and θ from 0.38 to 0.63. With the exception of θ, this data indicates a relatively wide range of fiber parameters covering a realistic range of fiber fineness but would require more immature values of θ ranging down to at least 0.25. As was mentioned in the Introduction, one of the reasons for this undertaking is that the measurement of Micronaire alone is not sufficient to predict fiber maturity. In Table II the data from Table I is sorted in the order of increasing maturity. We have also included the relative ranking of these specimens with respect to degree of thickening (maturity) θ. It is clear that there is a good deal of shuffling in the rank of the samples indicating that the highest Micronaire cottons are not necessarily the most mature cottons.

Validate the Image Analysis Software of Dr. Bugao Xu

One of the areas to check was the agreement between the Leica software which was the software originally used and a new more automated version known as the Fiber Image Analysis (FIA) system developed by Prof. Bugao Xu, U.T., Austin, TX. As discussed above, the Leica software allows for more individual manual editing and input while FIA is fairly automatic and makes use of algorithms to bypass the need for manual editing. To check agreement between the two approaches, we first considered a typical digital image of a cross section of a bundle containing approximately 60 fibers, which was analyzed by both the SRRC (Leica) software and Dr. Bugao Xu's (FIA) image analysis software. Comparisons were made between fiber-by-fiber measurements of area, perimeter, and theta. In Figure 1 we illustrate fiber-by-fiber correlation between fiber areas as measured by the Leica software and FIA. There is an excellent correlation of $R^2 = 0.957$ with a relatively small intercept (9.69) and slope (1.05) close to unity. In Figure 2 we illustrate fiber-by-fiber correlation between fiber perimeters as measured by the Leica software and FIA. There is an excellent correlation of $R^2 = 0.968$ with a relatively small intercept (3.23) and essentially unity slope (0.99). An illustration of the fiber-by-fiber correlation between fiber circularity (θ) as measured by the Leica software and FIAS is shown in Figure 3. There is an excellent correlation of $R^2 = 0.964$, but with a significant intercept (0.14) and non-unity slope (0.80). A comparison of the mean values of the three image analysis parameters as measured by Leica and FIA for the given in the set of data as shown in Table III. Leica and FIA agree best for the theta (θ) measurements with a difference between the mean values of 1.3 %. Their agreement is poorest for the area (μm^2) measurements with a difference of 14%. The agreement on the perimeter (µm) measure is intermediate at 6.4 (%).

Improve the Accuracy and Precision of the Method

We tried the approach with the 100 x oil-immersion objective, but the thickness of the boundary also increased proportionately so that the location of the boundary was not clarified any more than at the normal 40 x. We have decided that the best check of accuracy will be with the British Standard method applied to gravimetric fineness measurements. In the meantime, however, we did look into the precision of the image analysis method. One of the first pieces of information that was necessary in order to proceed with some confidence concerning the precision of the image analysis method was to verify what number of cross-sections needed to be measured per sample in order to guarantee precision or repeatability within certain statistical limits. The minimum number of samples that need to be analyzed for obtaining statistically valid, reproducible data was calculated using the following formula:

$$n = [(1.96)\{(\max - \min)/4\}]/B \qquad (1)$$

where:

n = the minimum number of samples to analyze; and
B = specified error of estimation for the mean

This experiment was carried out using three of the nine ITMF cottons (A2, B2, and C2 that were discussed in Thibodeaux 1998). Table IV indicates that a minimum sample size of 529 to 1436 fibers is needed to estimate the true population mean for each parameter with 99% confidence. Table V indicates the same analysis with 95% probability level and this indicates a sample size of less than 300 fibers is needed for determining the fiber parameters with 0.5% accuracy.

Future Plans

1. Use the ICCS blending, sample preparation protocol to prepare representative samples from the six bales of cotton from Texas Plains Cotton Cooperative representing three varieties with different levels of genetic fineness (perimeter) and a low and high level of maturity for each the three cotton types. Share samples between the three cooperating labs, SRRC, ITC, and Bremen and develop representative values for F/M.
2. Continue fundamental research to determine nature of variability of fiber perimeter within a sample of cotton. Basic question – Is the STD of the fiber perimeter distribution a result of fiber taper or does it reflect the normal variability of cotton? Will attempt to answer by comb sorting the sample before embedding and sectioning of sorted length group at the middle of the group (thus no tips or bases). Will also consider at least three length groups to ask question if there is a length bias to these measurements.
3. Begin to collect (to be done by Eric Hequet, ITC) large

amount of cottons (more than 100 samples) from world-wide contacts especially in Africa. Use IA techniques to characterize F/M for this diverse population and measure other fundamental physical properties.

4. Work to setting up a cooperative effort with the A.Y.S.A. so as to obtain samples of fabric with dyeing imperfections and develop a system whereby a selected samples of laydowns be archived so as to be able to be analyzed for maturity distributions after learning of dyeing problems.

References

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Table I. Range of maturity/fineness properties for experimental cultivars.

Cultivar	Area (μm)^2	Perimeter (μm)	Theta θ	Micronaire (measured)
Amsak	74 - 89	45 - 46	.45 - .56	2.6 - 3.1
ITMF-C	106 - 127	57 - 59	.38 - .47	2.7 - 4.1
DPL-15	115 - 123	56 - 58	.44 - .51	3.2 - 3.8
ITMF-A	103 - 114	53 - 54	.44 -.48	3.2 - 3.8
CA3084	97 - 111	48 - 51	.48 - .60	3.4 - 4.4
Giza-45	88 - 93	45 - 47	.52 - .58	3.5 - 3.9
ITMF-B	98 - 106	45 - 47	.54 - .58	3.9 - 4.3
Giza-75	106 - 129	48 - 52	.56 - .61	4.2 - 4.8
MAXXA	127 - 128	54 - 55	.54 - .56	4.4 - 4.8
SJ5	101 - 125	48 - 52	.57 - .59	4.7 - 4.8
ELDORADO	107 - 122	47 - 50	.62 - .63	4.7 - 5.0
SG-501	120 - 137	52 - 55	.57 - .6	4.9 - 5.4
DP-5415	138 - 144	52 - 57	.55 - .65	4.9 - 5.8
DPL-50	118 - 148	54 - 57	.54 - .57	5.2 - 5.5
SG-404	152 - 162	58 - 60	.54 - .59	5.4 - 6.2

Table II. An illustration of the fact that ranking cottons by Micronaire will not necessarily predict their relative maturity.

Cultivar	Ranking (Micronaire)	Ranking (Maturity)
Amsak	1	4
ITMF-C	2	1
DPL-15	3	2
ITMF-A	4	3
CA3084	5	5
Giza-45	6	6
ITMF-B	7	9
Giza-75	8	12
MAXXA	9	7
SJ5	10	13
ELDORADO	11	15
SG-501	12	14
DP-5415	13	11
DPL-50	14	8
SG-404	15	10

Table III. Differences in average image analysis parameters measured on the same 62 cross-sections by both the Leica and FIA systems.

Parameter	LEICA	FIA	Difference
(mean)			(%)
Area (m^2)	87.6	101.8	14.0
Perimeter (m)	42.0	44.9	6.4
Theta (θ)	0.63	0.64	1.3

Table IV. Number of fibers necessary to assess fiber quality parameters with 99% accuracy.

ITMF Cottons	Perimeter	Area	Theta
A2	1035	1436	800
B2	878	1156	529
C2	830	1154	862

Table V. Number of fibers necessary to assess fiber quality parameters with 95% accuracy.

ITMF Cottons	Perimeter	Area	Theta
A2	207	288	160
B2	176	231	106
C2	166	231	173

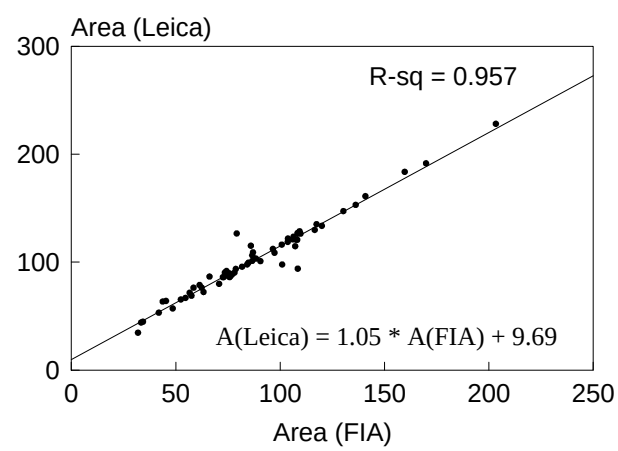

Figure 1. Fiber-by-fiber correlation between areas measured by Leica versus FIA image analysis software.

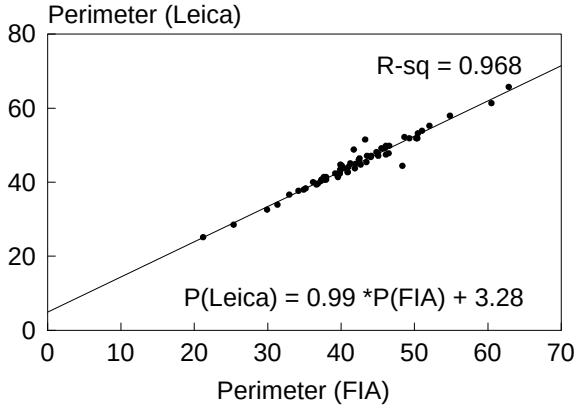

Figure 2. Fiber-by-fiber correlation between perimeters measured by Leica versus FIA image analysis software.

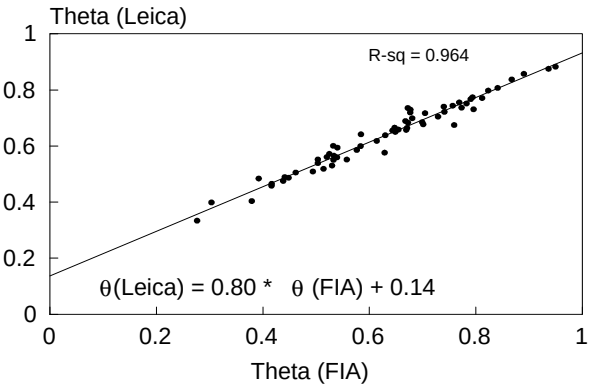

Figure 3. Fiber-byfiber correlation between θ measured by Leica versus FIA image analysis software.