NOTE

Development of New Reference Standards for Cotton Fiber Maturity

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INTERPRETIVE SUMMARY

The problem of correctly assessing the maturity (the degree of development of the fiber wall) of a sample of cotton remains a serious issue for the world textile industry. The present marketing system does not include a direct measure of maturity. The closest measurement to maturity currently available is micronaire, which measures a combination of fiber fineness and maturity.

This paper reports on the use of a reference technique for measuring cotton maturity, based on image analysis of thin fiber sections to develop a set of standard calibration cottons having a wide range of fineness and maturity values. We are working closely with several cotton geneticists/breeders, representing production of as diverse a set of cottons as possible, to develop a comprehensive set of cottons having a wide range of fiber maturities and fineness.

The image analysis reference method has been improved to the extent that it is giving reliable data with a minimum of problems and is at a point where the technology can be transferred to other laboratories. The technique for harvesting from specified zones on the plant has potential for obtaining cotton with a range of maturity values.

ABSTRACT

An accurate measure of the maturity of a sample of cotton is essential for assessing the quality of the fiber. Work has begun to produce large quantities of diverse cottons with well-defined values of fineness and maturity. 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. 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 methacrylate matrix, sectioning the bundle with a microtome, and microscopic image analysis to determine the maturity of each fiber in the thin sections. Findings of the research indicate that: (i) 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; (ii) 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; (iii) a reasonable validation has been established between the present reference method and the 1984 International Textile Manufacturers' Federation (ITMF) Round Test cottons; (iv) image analysis measurements of fiber cross-sectional area and perimeter can be used to predict micronaire; and (v) micronaire measurements alone are not good predictors of fiber maturity.

The problem of correctly assessing the maturity (the degree of development of the fiber wall) of a sample of cotton remains a serious issue for the world textile industry. The presence of immature cotton in a laydown poses significant problems in processing performance and in the quality of the finished textile. These problems may include: neps, weak places in yarns, ends-down in spinning, excess waste, and dyeing imperfections such as white specks and barré. The present marketing system does not include a direct measure of maturity. The closest

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Abbreviations: *A*, cell wall area (excluding lumen area) of fiber cross-section (μm^2); *P*, fiber perimeter (μm); θ, circularity or degree of thickening (defined as the ratio of the wall area *A* to the area of a perfect circle having perimeter *P*) (see. Eq. [1]); *MIC*, micronaire; M, maturity ratio.

measurement to maturity that is available is micronaire which, as will be demonstrated, measures a combination of fiber fineness and maturity.

The cotton fiber is a single cell formed by the elongation of an epidermal cell on the surface of the seed. Maturity refers to the degree of development or thickening of the fiber cell wall relative to the perimeter or effective diameter of the fiber. As seen in Fig. 1, the cell wall area (A) (excluding lumen) and perimeter (P) of the fiber cross-section are the two independent parameters that totally define the fiber's cross-sectional morphology. The circularity or degree of thickening, θ [defined as the ratio of the wall area A to the area of a perfect circle having perimeter P] is calculated from the equation

$$\theta = 4\pi A/P^2 \quad [1]$$

Since the *gravimetric fineness* or linear density of the fiber is directly proportional to the wall area (A) and the *genetic fineness* is related to the perimeter (P), the cross-sectional fiber measurement fully describes both cotton fiber maturity and fineness. Thibodeaux and Evans (1996) demonstrated that micronaire measures a combination of fiber fineness and maturity (see Equation 2) and is therefore not a totally reliable monitor of cotton maturity.

$$MIC = [8.56\,(A/P)^2 + 1.196]^{1/2} - 2.35 \quad [2]$$

Equation [2] allows the calculation of micronaire from microscopic measurements of cotton cross-sections. What is most significant here is that for a given MIC measurement there will be any number of combinations of A and P and therefore maturity (see Eq. [1]) that are possible.

Thibodeaux and Price (1988) reported on a method for maturity measurement based upon cross-sectioning a bundle of cotton fibers, mounting the section on a microscope slide, and measuring the sizes and shapes (A, P, and θ) of the sections with an image analysis computer system. Since developing and reporting on the method in the late 1980s, efforts have concentrated on improving sampling, preparation, embedding, sectioning, and microscopy (Boylston et al., 1995) to obtain thin sections having a larger number of individual (not touching) fiber cross-sections with excellent optical contrast (Fig. 2). In addition, Thibodeaux (1998) has reported an improved image analysis technique that is fast, accurate, and based upon an algorithm that allows for automated measurements of several hundred cross-sections per hour.

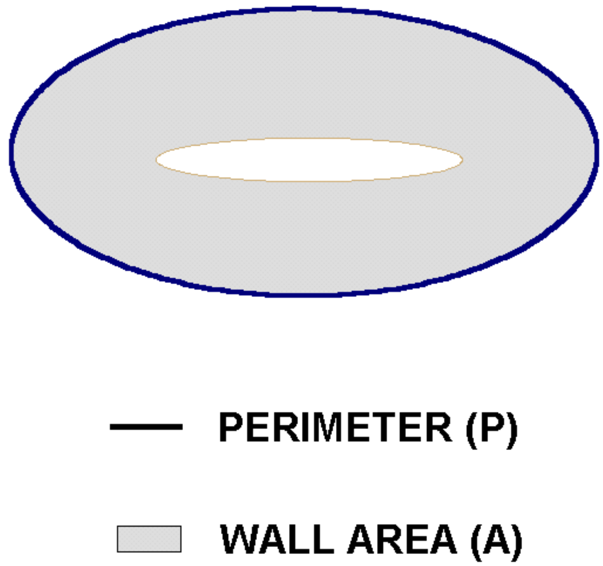

Fig. 1. Cross-section of a typical cotton fiber.

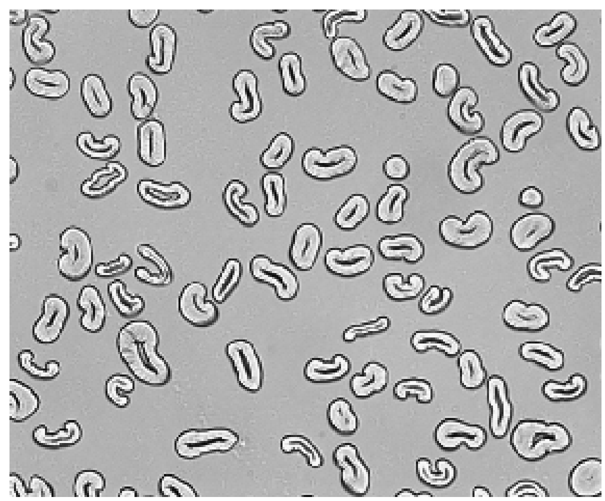
Fig. 2. Typical images of fiber cross-sections obtained with improved sample preparation methods described in this paper.

Because maturity is such an important parameter, it is desirable to include this measurement in routine fiber quality assessment. Several instruments are either on the market or under some stage of development to measure maturity – fiber/maturity tester (F/MT) Micromat (Gordon et al., 1997); advanced fiber information system (AFIS) (Peters, 1998); and near infrared or visible/near infrared reflectance spectrometers (Montalvo et al., 1987). The intent of this work has been to use the image analysis method to develop a set of well-blended cottons having a wide range of fineness and maturity values for use in the calibration and standardization of instruments currently in use and for assistance in developing new candidate methods for indirectly measuring the maturity of cotton. The purpose of this paper is to discuss initial efforts to develop these calibration cottons by enlisting the assistance of cotton geneticists/breeders representing a production of as diverse a set of cottons as possible to develop a comprehensive set of cottons having a wide range of fiber maturities and finenesses for calibration by the image analysis reference method.

MATERIALS AND METHODS

The model for the development of these sets of calibration cottons goes back to 1982–1983 when the ITMF, in cooperation with such organizations as the Bremen Fiber Institute, Hellenic Cotton Board, Egyptian Cotton Arbitration and Testing Organization, and Cotton Incorporated, developed a set of nine calibration cottons for use in large-scale maturity round trials. Three cotton varieties were selected representing a rather wide range of genetic finenesses. Within each variety three levels of maturity of fiber were obtained. Table 1 identifies the cottons and their relative maturity levels and perimeters grouped by their respective country of origin. It should be noted that the maturity ratio (M) is related to θ by

$$M = \theta / 0.577 \qquad [3]$$

The values of M were determined from F/MT using the average of several cooperating laboratories. Perimeters were determined by using planimeter analysis of micrographs of fiber cross sections. The A, B, and C cottons naturally divide into three groups, each with a fairly constant value of perimeter but different levels of M. The ideal set of maturity calibration cottons would ultimately require diverse genetic varieties (at least five) still having three maturity levels for each variety, but having a wider range of genetic finenesses or perimeters than the 1983 set.

Table 1 . Original data for the 1984 International Textile Manufacturers' Federation cottons.

CODE	Origin of Cotton	Maturity (M) [from F/MT]	Perimeter
			μm
B-2	MAK 73 —Greece	1.02	52.9
B-1	MAK 73 —Greece	0.96	52.4
B-3	MAK 73 —Greece	0.95	54.7
A-3	GISA 70 —Egypt	0.88	57
A-1	GISA 70 —Egypt	0.84	54.9
A-2	GISA 70 —Egypt	0.8	55.2
C-1	Upland—USA	0.87	64.4
C-3	Upland—USA	0.8	62.1
C-2	Upland—USA	0.67	59.8

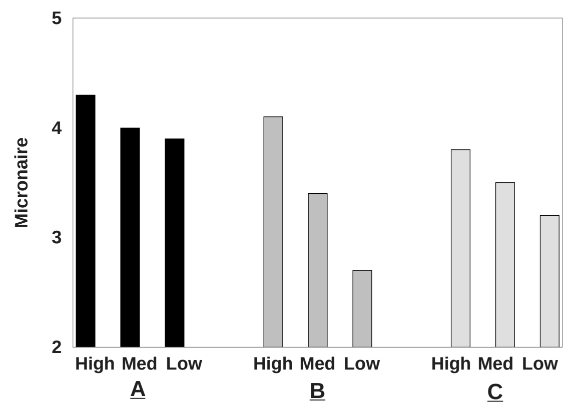

Fig. 3. Variation of micronaire for the three 1984 International Textile Manufacturers' Federation cottons, each with three levels of maturity.

The problem of obtaining an appropriate range of finenesses for a given variety can be addressed in two ways. The first involves harvesting from isolated zones on the cotton plant. In his presentation at the 1996 Engineered Fiber Selection Conference, Lewis (1996) explained an approach to dividing a plant into six zones based upon fruiting position, which includes segregation based upon location of the branch on the plant and of position of the boll out on the branch. He obtained a micronaire range between 3.4 and 4.8 with a strong relationship between micronaire and plant zone.

The second approach, involves the concept discussed with Eq. [2], wherein if cotton bales of known variety were identified, then the fiber perimeter should be reasonably constant and micronaire should be a good predictor of wall area and, thus, maturity. To illustrate this principle, consider the chart illustrated in Fig. 3, where the values of micronaire for the three groups of ITMF cottons are shown. Thus for each cultivar, the different micronaire levels are indicative of the varying levels of maturity for each of the three cottons, validating the discussion of Eq. [2].

Several U.S. cotton breeders have agreed to cooperate in order to obtain these samples. The cottons were obtained by hand-picking bolls from various locations on the plant to give the desired variations in maturity. Thus far, the cooperators have included (going from west to east): Drs. John Pellow and H.B. Cooper, Phytogen Seeds, Boswell Corp., Corcoran, CA; Dr. Richard Percy, USDA-ARS, Maricopa, AZ; and Dr. John Gannaway, Texas A&M, Lubbock, TX.

Approximately 50 varieties representing different cotton species and a wide range of genetic finenesses were grown and hand-harvested during 1997. In general, sample sizes ranging from 50 to 200 gms were received and screening has begun. For the purposes of this paper, results from a subset of 12 of these cultivars will be presented with image analysis and micronaire data obtained from samples harvested from the top, middle, and bottom zones of the plant. Subsamples of these fibers were obtained and prepared by embedding and sectioning prior to image analysis.

The image analysis system used was a Leica Model 600 interfaced to a Nikon Optiphot POL light microscope operating in the transmitted mode. An image-analysis routine was developed that measured the values of A and P for all cross sections in 20 individual fields of view of the 1 µm thick section. The dimension of the measuring frame was about 185 µm by 140 µm with a resolution of 0.338 µm per pixel. There were typically 30 to 50 cross sections per field.

RESULTS AND DISCUSSION

As mentioned above, initial efforts were aimed at the testing of the ITMF reference cottons. Results for average area, perimeter, and maturity ratio calculated from Eq. [3] using the values obtained with image analysis of the ITMF reference cottons are summarized in Table 2. The values of the maturity ratio from the original round test data for the F/MT (Table 1) were compared with the calculated values for M (Table 2). Analysis of regression between these two data sets yields the graph in Fig. 4. Results are excellent, with a high coefficient of determination ($R^2 = 0.88$), a slope near unity (0.994), and an intercept close to 0 (0.0183). This level of agreement between the present reference method and the results obtained some 16 years ago establishes an important link and validation of the approach.

Table 2. Results of recent image analysis of the 1984 International Textile Manufacturers' Federation cottons.

CODE	Area	Perimeter	Maturity (M) [from Eq. [3]]
	μm^2	μm	
B-2	98.77	44.83	0.96
B-1	100.47	45.04	1.02
B-3	105.93	47.03	0.95
A-3	106.29	53.16	0.82
A-1	114.25	54.28	0.84
A-2	102.54	53.86	0.77
C-1	127.17	57.64	0.83
C-3	113.42	57.03	0.76
C-2	105.9	58.6	0.67

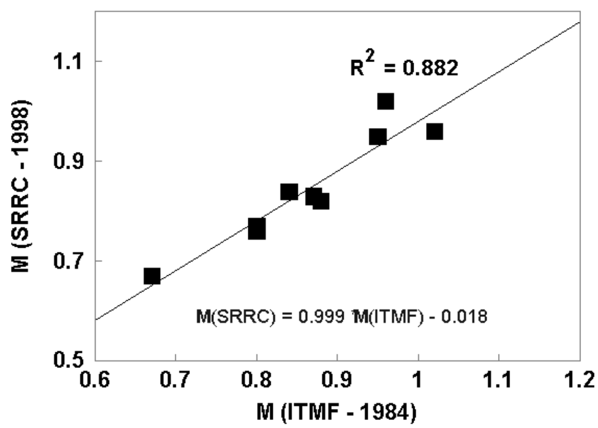

Fig. 4. Relationship between maturity ratio (M) determined by present-day image analysis and the original results from the 1984 International Textile Manufacturers' Federation round trials obtained with the Fineness/Maturity tester.

Results obtained with the subset of the 12 cultivars planted and hand-harvested by the collaborators are shown in Table 3, where there has been included the cultivar name, and the ranges of the average values of fiber wall area, perimeter, and degree of thickening (θ), all of which were determined from image analysis. 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.

Table 3. Range of maturity/fineness properties for experimental cultivars ranked by increasing micronaire.

Rank (Micronaire)	Cultivar	Area	Perimeter	θ	Micronaire (measured)
		μm^2	μm		
1	Amsak	74–89	45–46	0.45-0.56	2.6–3.1
2	ITMF-C	106–127	57–59	0.38–0.47	2.7–4.1
3	DPL-15	115–123	56–58	0.44–0.51	3.2–3.8
4	ITMF-A	103–114	53–54	0.44–0.48	3.2–3.8
5	CA3084	97–111	48–51	0.48–0.60	3.4–4.4
6	Giza-45	88–93	45–47	0.52–0.58	3.5–3.9
7	ITMF-B	98–106	45–47	0.54–0.58	3.9–4.3
8	Giza-75	106–129	48–52	0.56–0.61	4.2–4.8
9	MAXXA	127–128	54–55	0.54–0.56	4.4–4.8
10	SJ5	101–125	48–52	0.57–0.59	4.7–4.8
11	Eldorado	107–122	47–50	0.62–0.63	4.7–5.0
12	SG-501	120–137	52–55	0.57–0.6	4.9–5.4
13	DP-5415	138–144	52–57	0.55–0.65	4.9–5.8
14	DPL-50	118–148	54–57	0.54–0.57	5.2–5.5
15	SG-404	152–162	58–60	0.54–0.59	5.4–6.2

Table 4. Range of maturity/fineness properties for experimental cultivars ranked by increasing maturity (θ).

Rank (Micronaire)	Cultivar	Area	Perimeter	θ	Micronaire [from Eq. [2]]	Micronaire (measured)
		μm^2	μm			
2	ITMF-C	106–127	57–59	0.38–0.47	3.1–4.2	2.7–4.1
3	DPL-15	115–123	56–58	0.44–0.51	3.2–3.8	3.2–3.8
4	ITMF-A	103–114	53–54	0.44–0.48	3.3–3.9	3.2–3.8
1	Amsak	74–89	45–46	0.45–0.56	2.6–3.1	2.6–3.1
5	CA3084	97–111	48–51	0.48–0.60	3.5–4.1	3.4–4.4
6	Giza-45	88–93	45–47	0.52–0.58	3.3–3.8	3.5–3.9
9	MAXXA	127–128	54–55	0.54–0.56	4.5–4.7	4.4–4.8
14	DPL-50	118–148	54–57	0.54–0.57	4.2–5.0	5.2–5.5
7	ITMF-B	98–106	45–47	0.54–0.58	4.2–4.3	3.9–4.3
15	SG-404	152–162	58–60	0.54–0.59	5.2–5.8	5.4–6.2
13	DP-5415	138–144	52–57	0.55–0.65	4.8–5.5	4.9–5.8
8	Giza-75	106–129	48–52	0.56–0.61	4.0–5.1	4.2–4.8
10	SJ5	101–125	48–52	0.57–0.59	3.9–4.8	4.7–4.8
12	SG-501	120–137	52–55	0.57–0.6	4.5–5.1	4.9–5.4
11	Eldorado	107–122	47–50	0.62–0.63	4.4–4.9	4.7–5.0

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 a range of fineness/maturity values as did the ITMF cottons. Although 15 cultivars are represented here, the table represents results obtained with three times that many 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 μm^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 theses is that the measurement of micronaire alone is not sufficient to predict fiber maturity. In Table 4 the data from Table 3 is sorted in the order of increasing 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. Also included in Table 4 is a column giving the micronaire calculated using the values of given for A and P and calculated using Eq. [2]. The values of the two ranges placed side-by-side for each cultivar appear to be quite close. In Fig. 5, the measured micronaire values have been plotted against the micronaire calculated from the image analysis area and perimeter (Eq. [2]). Results are quite

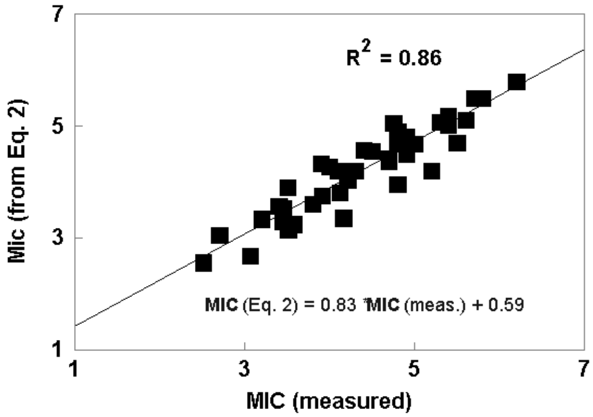

Fig. 5. Relationship between measured micronaire and micronaire predicted from Eq. [2].

respectable, with a good coefficient of determination (R^2 = 0.86), a slope of 0.86 and an intercept of 0.59.

CONCLUSIONS

The image analysis reference method has been improved to the extent that it is giving reliable data with a minimum of problems and is at a point where the technology can be transferred to other laboratories.

Producing a range of fiber maturities by selective harvesting from only three zones on the plant will give reasonable differences in fiber area, perimeter, and micronaire, but only marginal differences in maturity.

A reasonable link and validation has been established between the present reference method and the 1984 ITMF Round Test cottons.

It is possible to use image analysis measurements of fiber cross-sectional area and perimeter to predict the micronaire of a sample of cotton.

Micronaire measurements alone are not good predictors of fiber maturity.

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