Evaluating Cell Wall Structure and Composition of Developing Cotton Fibers Using Fourier Transform Infrared Spectroscopy and Thermogravimetric Analysis

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ABSTRACT: Universal attenuated total reflectance Fourier transform infrared (UATR-FTIR) spectroscopy and thermogravimetric analysis (TGA) were used to investigate the structural changes of cotton (*Gossypium hirsutum* L.) fibers as a function of developmental programming. The presence of noncellulosic compounds (wax, protein, hemicelluloses, pectic substances, amino acids, etc.) was evident from FTIR spectra of fibers at 10, 14, 17, and 20 dpa (day postanthesis). The vibration corresponding to the noncellulosic compounds disappeared at 36 dpa. Furthermore, independent TGA analysis supported the results obtained with FTIR, showing that the transition from primary cell wall synthesis to secondary cell wall synthesis occurs at or around 20 dpa. This study is the first to report on the use of the UATR-FTIR and TGA to elucidate structural changes during cotton fiber development. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 476–486, 2008

Key words: cotton; cell wall; fiber development; *Gossypium*; FTIR; TGA

INTRODUCTION

Fourier transform infrared spectroscopy (FTIR) has emerged as a key technique for the study of plant growth and development. Zeier and Schreiber used FTIR to characterize isolated endodermal cell walls from plant roots and assigned FTIR frequencies to functional groups present in the cell wall, including the relative amounts of the cell wall biopolymers suberin and lignin, as well as cell wall carbohydrates and proteins.[1] FTIR absorption spectra indicated structural differences for three developmental stages of the endodermal cell wall under study. The authors concluded that FTIR could be used as a direct and nondestructive method suitable for the rapid investigation of isolated plant cell walls. The approach has since been successfully applied to screen large numbers of mutants for a broad range of cell wall phenotypes using FTIR of leaves of *Arabidopsis thaliana* and flax (*Linum usitatissimum*).[2] In this study, Chen et al. reported that principal component analysis (PCA) of FTIR spectra can distinguish between mutants that are deficient in cell wall sugars. Also, FTIR micro-spectroscopy has been used to determine the presence of functional groups in cellulose and pectin molecules within an individual plant cell wall.[3–5]

Thermogravimetric analysis (TGA) is one of the most commonly used thermal techniques for the characterization of both inorganic and organic materials, including polymers (such as cellulose). It provides quantitative results regarding the loss of weight of a sample as a function of increasing temperatures. It has previously been observed that the major weight loss of the developing cotton fibers occurs between 130 and 380°C.[6] Moreover, TGA measurements provide basic information about the thermal properties of the material and its composition. The derivative thermogravimetry can be used to investigate the differences between thermograms. This technique was used, for example, to elucidate differences in flax fibers and determine their fineness.[7] Abidi et al.[8] investigated the relationships between three important cotton fiber physical properties—micronaire, maturity, and fineness—and fiber thermal properties as determined by TGA. The results showed significant effects of the cotton fiber type on the percent weight loss and the decomposition temperatures of cellulose in the temperature region 225–425°C. In this region, good correlations were established between the weight loss and the quantity of primary cell wall per unit mass. Differences

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in weight loss between two cottons having different maturities allowed the authors to estimate the primary cell wall width that is in agreement with the existing literature.

Cotton fiber development consists of five major overlapping developmental stages[9]: differentiation, initiation, polar elongation, secondary cell wall deposition, and maturation. Fiber initiation, which commences at anthesis (0 days postanthesis = 0 dpa), signals the onset of fiber morphogenesis. During the period of rapid polar elongation, fiber growth is characterized by the synthesis of the primary cell wall and an increase in fiber length up to $\sim$ 30 mm within 3 weeks after anthesis. The stage of secondary cell wall growth, which commences around 21 dpa and continues for a period of $\sim$ 3–6 weeks postanthesis, is marked by the massive deposition of a thick cellulosic wall.[9] The transition period between 16 and 21 dpa is considered to represent a developmental switch in emphasis from primary to secondary cell synthesis during cotton fiber development. During these developmental stages, important structural changes occur leading to cellulose macromolecules formation (β (1 $\rightarrow$ 4) glucopyranose).

Huwyler et al. reported on the changes in the composition of cotton fiber cell walls during development.[10] The authors performed successive extractions of purified cell walls, prepared from cotton fibers at different growth stages. The authors showed that the most noticeable change was the decrease in the absolute amounts of noncellulosic glucose and arabinose residues during the secondary cell wall formation. Timpa and Triplett using gel-permeation chromatography analyzed cell wall polymers during cotton fiber development.[11] Among their findings, the authors showed that high-molecular weight materials decreased during the period of 10–18 dpa with concomitant increase in lower molecular weight wall components. The authors attributed this behavior to a possible hydrolysis during the later stage of elongation. Other studies were conducted aimed at investigating the biochemical composition of the cell wall of the cotton fibers during development.[12–15] However, they are all based on cell wall extraction followed by time consuming analysis.

To study the structural changes and gain novel insight into cell wall structure and composition in developing cotton fibers, we used FTIR and TGA. Fiber samples were collected at different stages of fiber development (days postanthesis) and analyzed.

EXPERIMENTAL

Materials

For this study, three replications of two cotton cultivars (*Gossypium hirsutum* L. cv. TX19 and TX55) were planted in a greenhouse on October 21st 2005 with day/night cycles varying from 13/11 to 11/13 h and day/night temperatures of about 31°C/24°C. Plants were grown in 20 L (5 gallons) pots of Sungrow SB 300 potting mix that had been amended with Peters 15-9-12 slow release fertilizer prior to potting. Plants were watered as needed. On the day of flowering (0 dpa), individual flowers were tagged, and five developing bolls per cultivar and per replication were harvested at 10, 14, 17, 20, 36, 50, and 61 dpa. The pericarp was immediately removed (excised with scalpel) and isolated ovules were transferred to cryogenic vials and stored in a Cryobiological Storage System filled with liquid nitrogen for FTIR and TGA analyses. Each replication was tested independently.

Methods

Sample dehydration

An established dehydration procedure for frozen samples was carried out as previously described.[16,17] The dehydration procedure consists of washing the hydrated sample (previously rinsed with water) with acidified 2,2-dimethoxypropane (one drop of HCl in 50 mL of 2,2-dimethoxypropane), followed by five exchanges for 15 min each in 100% acetone. In a slightly acidic solution, 2,2-dimethoxypropane is instantly hydrolyzed by water to form methanol and acetone.[16,18]

FTIR measurements

Spectrum-One equipped with an UATR (Universal Attenuated Total Reflectance) accessory (Perkin–Elmer, USA) was used to record FTIR spectra of the cotton fiber samples in an environmentally-controlled laboratory maintained at 65% $\pm$ 2%RH and 21°C $\pm$ 1°C. The universal attenuated total reflectance Fourier transform infrared (UATR-FTIR) was equipped with a ZnSe-Diamond crystal composite that allows collection of FTIR spectra directly on a sample without any special sample preparation. The instrument is equipped with a ''pressure arm'' which is used to apply a constant pressure to the cotton samples positioned on top of the ZnSe-Diamond crystal. The amount of pressure applied is monitored by the Perkin–Elmer FTIR software. This ensures good contact between the sample and the incident IR beam and prevents the loss of the IR beam.

Thirty FTIR spectra per sample were acquired for each developmental stage to produce a total of 90 spectra (30 spectra $\times$ 3 replications). All FTIR spectra were collected at a spectrum resolution of 4 cm^{-1}, with 32 coadded scans over the range from 4000 to 650 cm^{-1}. A background scan of clean ZnSe-Diamond crystal was acquired before scanning the

samples. The Perkin–Elmer software was used to perform baseline corrections of the spectra.

FTIR spectra analysis

FTIR spectra were normalized and subjected to PCA with leverage correction and mean-center cross validation boxes checked using Unscrambler V. 9.6 Camo Software AS (CAMO Software AS, Norway).

TGA

TGA of fiber samples was performed using the Pyris 1 TGA equipped with a 20-sample autosampler (PerkinElmer Shelton, CT). Thermograms were recorded between 37 and 600°C with a heating rate of 10°C/min in a flow of nitrogen at 20 mL/min.

Cotton lint samples were rolled into small balls (between 1.5 and 2 mg) by hand (wearing latex gloves to avoid moisture transfer), and then placed on the sample pan. Three replications were performed for each cotton sample. The Pyris software was used to calculate the first derivatives of the thermograms and to determine the percent weight loss for each sample.

Scanning electron microscope and fiber cross-sections

Scanning electron microscopy of the cotton fibers was performed using the Hitachi Microscope TM-1000 using an accelerating voltage of 15 kV. Fibers were placed on a carbon disc and no coating was performed prior to testing.

Fiber cross sections were performed according to the protocol reported in.[19] The fiber samples were embedded with a methacrylate polymer. This polymer holds the cotton fibers until they can be glued to a slide for observation. Then, the methacrylate polymer is dissolved in methyl ethyl ketone. After the slides are prepared, the images are viewed with a microscope and captured. Finally the image files are analyzed by the FIAS software.[20] The determination of the wall thickness for fibers 20 dpa and younger was not possible due to the lack of secondary cell wall in those fibers.

RESULTS AND DISCUSSION

Fiber maturity is a major yield component and an important fiber quality trait that is directly linked to the quantity of cellulose deposited during secondary cell wall biogenesis, and to the organization and orientation of crystalline microfibrils. It is inuitively obvious to hypothesize that immature fibers (having a thin, poorely developed secondary wall) will be fragile, and therefore, are likely to break during the multiple mechanichal stresses involved in transforming fibers into yarns. Immature fibers generate short fibers and neps (entranglement of fibers) that result in yarn defects and decreased productivity in the spinning mills. Therefore, studying cotton fiber maturity and understanding the link between secondary cell wall biogenesis and cotton fiber maturity is very important. This study was designed to investigate the structural changes occurring during the growth and development of cotton fibers.

FTIR analysis

A series of FTIR spectra of cotton fiber samples from the cultivar TX19 at 10, 14, 17, 20, 36, 50, and 61 dpa developing stages is shown in Figure 1. A spectrum of a mature cotton fiber sample is also shown as a control. Assignments of all observed bands in the spectra of developing cotton fibers and mature cotton are summarized in Table I.

Vibrations located at $\sim$ 2918 and $\sim$ 2850 cm^{-1}

These vibrations are attributed to $—CH_2$ asymmetric stretching and could originate from the wax substances present on the surface (cuticle) of developing fibers. The intensities of these two peaks start decreasing when the developing fibers reach 20 dpa; the stage representing the termination of primary cell wall synthesis and fiber elongation, and the early stage of secondary cell wall synthesis. In young fibers (<20 dpa), the primary cell wall is dominant and contains between 35 and 50% of cellulose.[10] The remaining components of the primary cell wall are waxes, pectins and other noncellulosic polysaccharides. The percent contribution of the primary cell wall to the total weight of the fiber versus secondary cell wall thickness was calculated using the following data: fiber diameter = 14 μm, density of cellulose = 1.52 g cm^{-3}, density of the primary cell wall = 1.14 g cm^{-3},[8] thickness of the primary cell wall = 0.4 μm. Figure 2 shows that the percent contribution of the primary cell wall to the total weight of the fiber decreases as wall thickness value increases (increased fiber maturity). Therefore, the relative importance of the vibration bands attributed to noncellulosic substances and located essentially in the primary cell wall is less because of the development of the secondary cell wall. This could explain why some vibration bands decrease after 20 dpa.

Vibration located at 1737 cm^{-1}

This vibration is attributed to C=O stretching and could originate either from esters, fatty acids or amides.[6] This vibration is very strong in the spectra

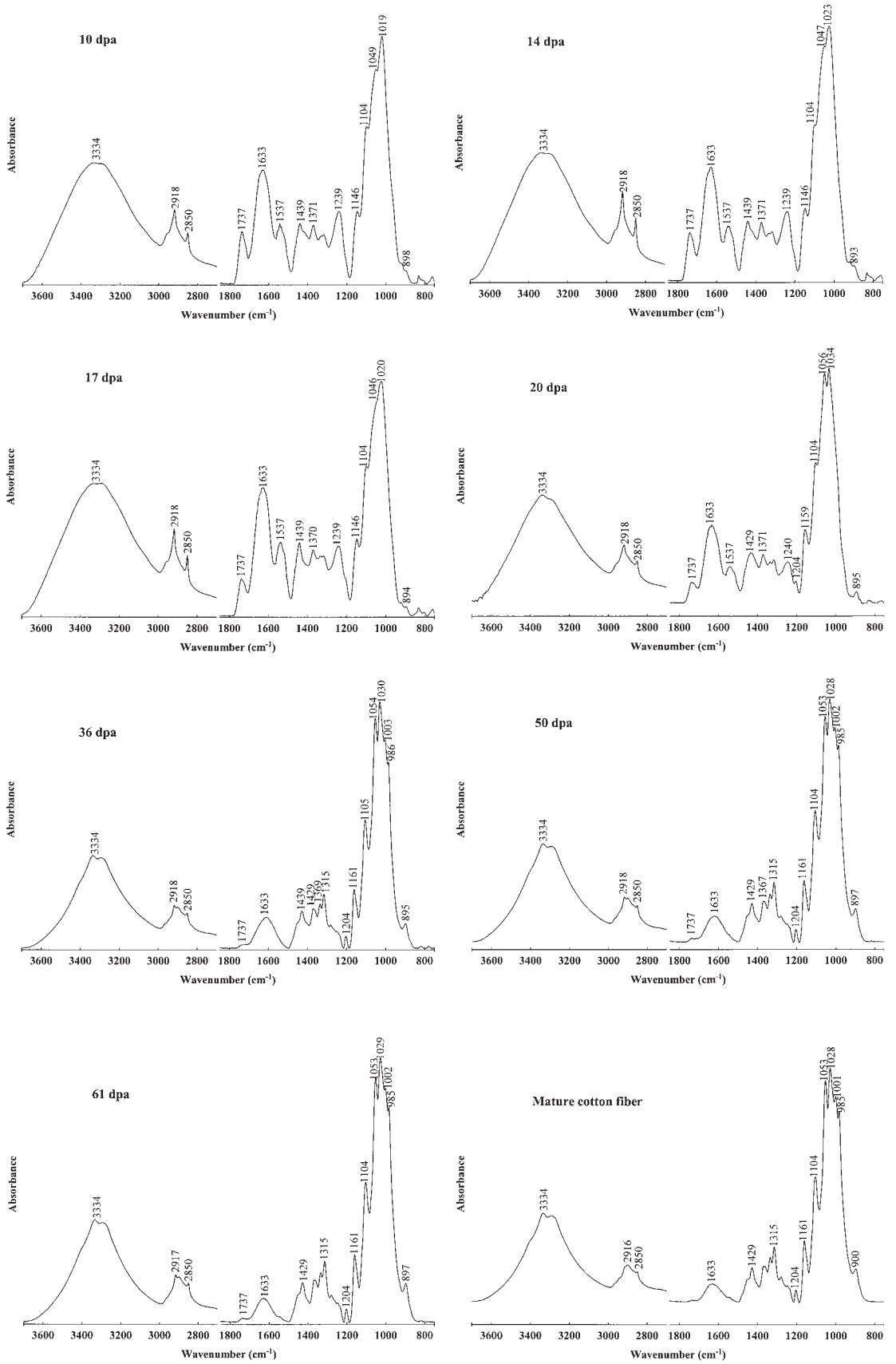

Figure 1 FTIR spectra of developing cotton (*Gossypium hirsutum* L. cv. TX19) fibers at different days postanthesis (dpa).

TABLE I
IR Assignments of the Main Vibrations in FTIR Spectra of Developing and Mature Cotton Fibers

Wavenumber (cm^{-1})	Tentative assignment
~ 3334	O—H stretching (intermolecular hydrogen bonds)
~ 2918 and ~ 2850	Asymmetric CH_2 stretching
~ 1737	C=O stretching
~ 1633	Adsorbed H_2O
~ 1537	NH_2 deformation (amide II)
~ 1439	CH_2 scissoring
~ 1429	CH_2 symmetric deformation
~ 1371	C—H bending
~ 1245	C=O stretching or NH_2 deformation (amide II)
~ 1204	COH in-plane bending mode
~ 1161	Antisymmetric bridge $C_{(1)}$—O—$C_{(4)}$ stretching mode
~ 1104	Asymmetric ring stretching mode
~ 1053	C—O stretch
~ 1028	C—C stretch
~ 900	β-linkage

of young fibers at 10, 14, and 17 dpa and starts decreasing in intensity at 20 dpa. The dominant constituents of primary cell walls of growing cotton fibers are polysaccharides such as acidic polymers, β-1,3-glucans and xyloglucans.[10,14] The amount of noncellulosic compounds increases during the fiber elongation stage (up to 20 dpa) and starts decreasing thereafter, while the amount of cellulose increases dramatically after the elongation stage.[13] Therefore, this vibration could be used to illustrate the end of the elongation phase and the beginning of secondary cell wall synthesis phase.

Vibration located at ~ 1633 cm^{-1}

This vibration is attributed to adsorbed water. It is noteworthy that this band diminishes in intensity with increasing dpa. This suggests that this band originates from adsorbed water molecules via hydrogen bonding in the amorphous regions of the cellulose macromolecules.[21] The decrease in the intensity during fiber development is due to an increase in the internal organization of the cellulosic chains allowing the formation of highly crystalline regions.

Vibration located at ~ 1537 cm^{-1}

This vibration is attributed to NH_2 deformation (belonging to amide II), and likely indicative of proteins or amino acids. It disappears completely when the developing fibers reach 36 dpa. At 36 dpa, the wall thickness is 1.84 μm for TX19 and 2.03 μm for TX55. Therefore, it is likely that the IR beam is not penetrating into the lumen of the cotton fibers. Thus, the proteins and amino acids present in the vacuoles of the developing fibers would not be detected.

Vibration at ~ 1439 and 1429 cm^{-1}

The vibration at 1439 cm^{-1}, attributed to CH_2 scissoring, may represent the presence of wax. Beginning at 20 dpa, this vibration weakens and a vibration located around ~ 1429 cm^{-1} appears. It is attributed to CH_2 symmetric deformation. O'Connor et al.[22] designated this band as a crystalline cellulose absorption band. The authors showed that the intensity of the band decreased and eventually disappeared when cellulose was decrystallized. Therefore, the appearance of the vibration 1429 cm^{-1} at 20 dpa could indicate that, in addition to the beginning of the cellulose synthesis in the secondary cell wall, structural organization (crystallinity) of the cellulose macromolecules occurs. This finding is in agreement with previous work.[23] Hsieh et al. reported that ''cellulose I crystalline structure is clearly evident at an early developmental stage of 21 dpa''. The weakening of the vibration located at 1439 cm^{-1} when the fibers reach 20 dpa could be attributed to the fact that the primary cell wall becomes less dominant.

Vibration located at 1204 cm^{-1}

This vibration is attributed to COH in-plane bending mode.[21] This vibration appears as a small shoulder at 20 dpa and is more pronounced at 36 dpa and thereafter. This vibration appears along with the vibration at 1429 cm^{-1}. Thus, 1204 and 1429 cm^{-1} could be both due to structural organization of the

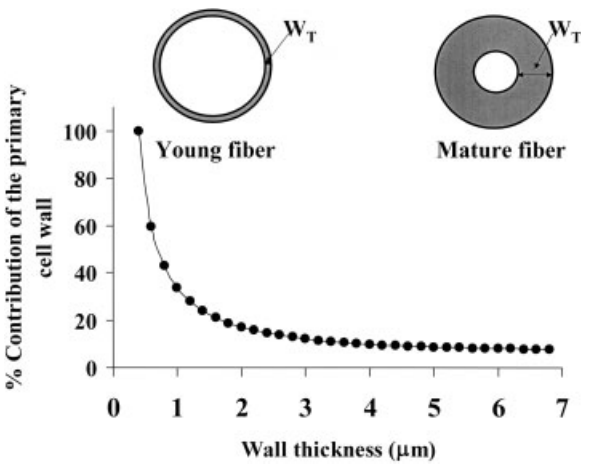

Figure 2 Relationship between the percent contribution of the primary wall to the total weight of the fiber and wall thickness W_T (calculation made for fiber diameter equals to 14 μm, density of cellulose = 1.52 g cm^{-3}, density of primary cell wall = 1.14 g cm^{-3}, and thickness of the primary cell wall = 0.4 μm).

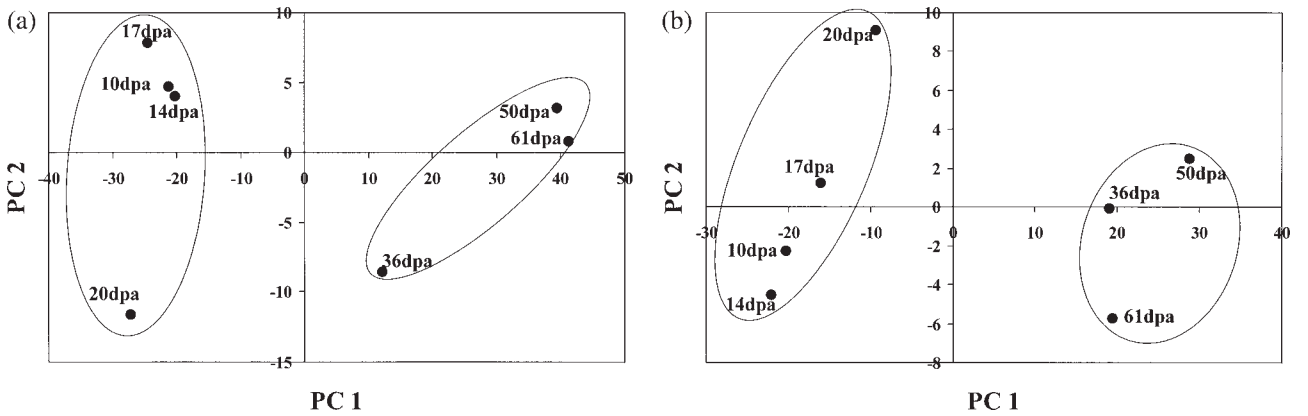

Figure 3 (a) Principal component analysis of the FTIR spectra (TX19). (b) Principal component analysis of the FTIR spectra (TX55).

cellulose during fiber development and could be used as secondary cell wall fingerprint.

Vibrations located at 1161, 1146, 1104, 1053, 1029 cm^{-1}

The vibrations 1146, 1104, and 1046 cm^{-1} are present only as small shoulders in spectra of developing cotton fibers at 10, 14, and 17 dpa. At 20 dpa, these vibrations become sharp and more define. The vibration 1146 cm^{-1} is shifted to 1161 cm^{-1}. As reported by several groups,[9,14] the deposition of cellulose during the secondary cell wall synthesis in cotton fibers begins around 21 dpa. Therefore, it seems reasonable to hypothesize that the vibrations located at 1161, 1104, and 1053 cm^{-1} are associated with the secondary cell wall synthesis.

Vibration located at $\sim$ 900 cm^{-1}

This vibration, attributed to β (1 → 4) linkage (glucosidic bond between two glucose units), appears only when the fiber reaches 20 dpa. From 10 to 20 dpa, this vibration is present only as a very small shoulder. The switch from primary cell wall synthesis to secondary cell wall synthesis (cellulose synthesis) is associated with strong vibration at 900 cm^{-1} (higher concentration of glucosidic bonds). This indicates that the condensation reactions between glucose units have been initiated which leads to the formation of β (1 → 4) glucopyranose macromolecules (cellulose).

PCA of FTIR spectra

PCA was performed to identify distinct groups of spectra.[2,24] PCA is a widely used mathematical technique to reduce the dimensionality of the data from several hundred variables (wavenumbers) in the original spectra to a fewer number of dimensions.[2] The variability in each spectrum relative to the mean of the population can be represented as a smaller set of values (axes) termed principal components (PCs). The effect of this process is to concentrate the sources of variability in the data into the first few PCs. Plots of PC scores against one another can reveal clustering in the data set. The plots of the PC1 versus PC2 scores are depicted in Figure 3(a,b), respectively, for TX 19 and TX 55. The FTIR spectra fall into two distinct clusters: Group 1 includes spectra of fibers at 10, 14, 17, and 20 dpa; while Group 2 includes spectra of fibers at 36, 50, and 61 dpa. PC1 accounted for 94% of the total spectra variation and could be used to separate the two groups.

Scanning electron microscopy and image analysis of fiber cross-sections

Scanning electron microscopy micrographs of developing cotton fibers are shown in Figure 4. For fibers at 10, 14, and 17 dpa the fiber walls are extremely thin and the fibers are stuck together. Therefore, the bundles of fibers could not be separated into individual fibers. At 20 dpa, the fibers can be individualized. They are composed essentially of primary cell wall and small amount of secondary cell wall. Beginning at 36 dpa, the appearance of twist indicates the presence of a thicker secondary cell wall as demonstrated by the fiber cross section measurements.

Fiber cross-section images at 20, 36, and 61 dpa shown in Figure 5(a), illustrate the developmental changes to the fiber cell walls. Cross-sections of fibers at 20 dpa show mostly the thin primary cell wall, while the secondary cell wall is not yet detectable. At 36 dpa, secondary cell wall thickening is clearly visible, demonstrating that secondary cell wall synthesis is well underway at this stage. By 61 dpa, the fiber cross-section shows a thick secondary cell wall indicating that the fibers have reached full maturity. Table II summarizes the results of the image analysis of fiber cross-sections. The evolution

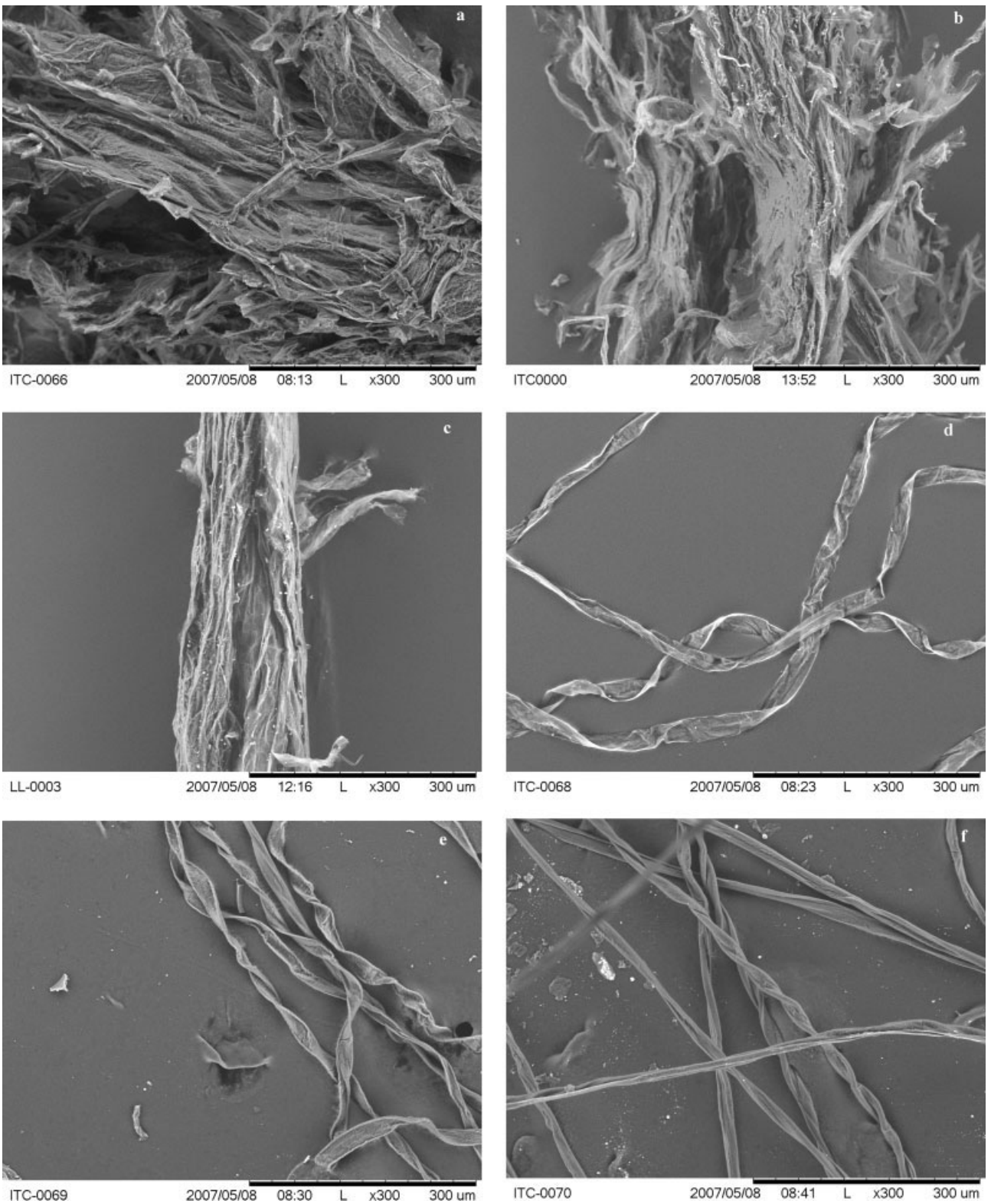

Figure 4 Scanning electron microscopy micrographs of developing cotton fibers (TX19): (a) 10 dpa, (b) 14 dpa, (c) 17 dpa, (d) 20 dpa, (e) 36 dpa, and (f) 50 dpa.

of the cell wall thickness as function of days postanthesis is shown in Figure 5(b). As mentioned before, we were not able to measure the cell wall thickness of fibers younger than 36 dpa.

TGA

TGA was performed on developing cotton fibers as described in the materials and methods section. The

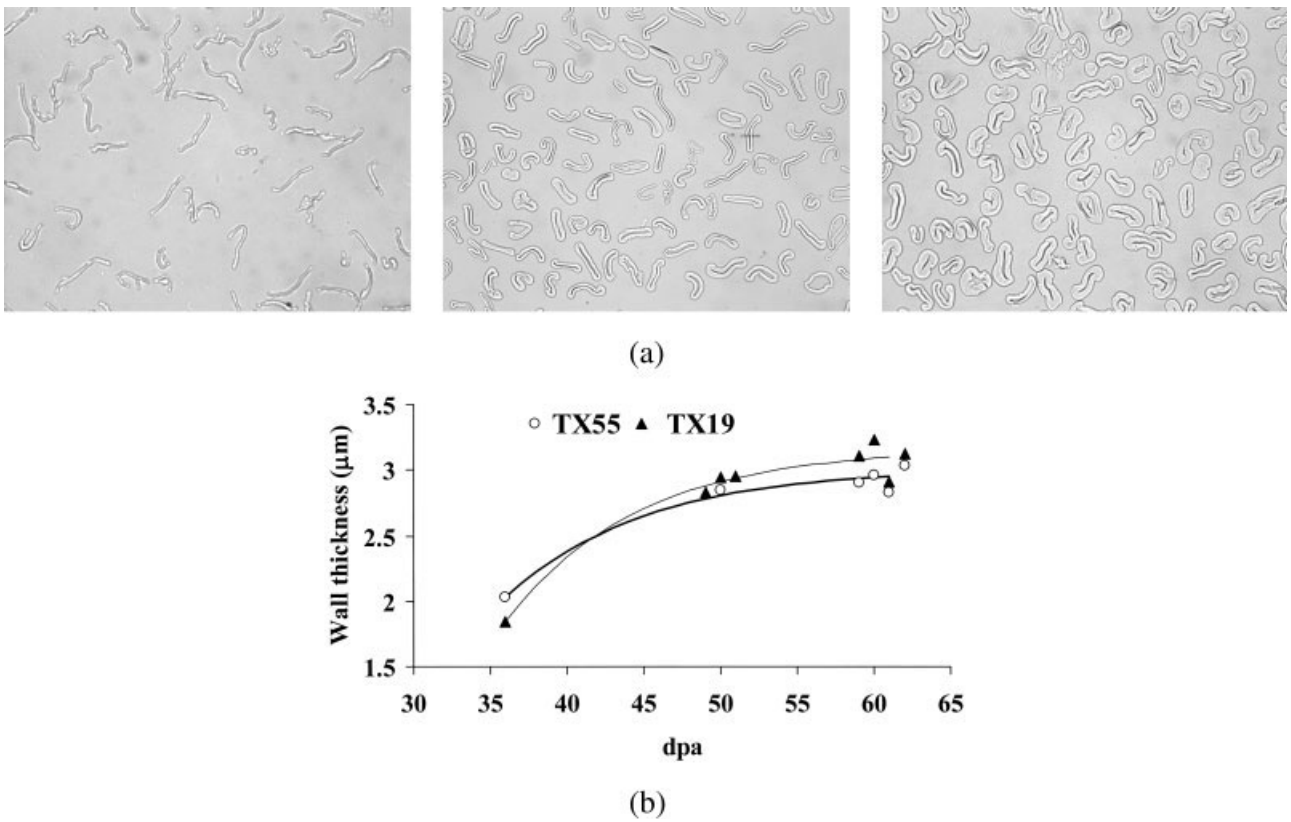

Figure 5 (a) Cross-section of fibers (TX19) at different development stages (left: 20 dpa; middle: 36 dpa; right: 61 dpa). (b) Wall thickness versus days postanthesis for TX19 and TX55.

goal is to assess the structural organization and evolution during fiber development. Noncellulosic compounds having low decomposition temperature would degrade first and this would translate into multiple thermal transitions in the thermogram.

Percent weight loss as a function of the temperature was recorded for each sample. The initial weight loss occurs between 37 and 150°C followed by a major weight loss between 150 and 450°C (Fig. 6). The first weight loss is due essentially to adsorbed water molecules, while the second weight loss is attributed to the decomposition of noncellulosic and cellulosic materials. There is a third weight loss between 450 and 600°C attributed to the loss of the product of decomposition in the second region (150–450°C).

Weight loss between 37 and 150°C

Statistical analysis (analysis of variance) showed a significant effect of the development stage (dpa) on the percent weight loss in both regions (Table III). The effect of the cultivar was not statistically significant. The average values of the percent weight loss for TX19 and TX55 as function of days postanthesis are illustrated in Figure 7. There are no significant differences between the percent weight loss of fibers at 10, 14, 17, and 20 dpa in this region. However, a reduction in the percent weight loss from 10% to 5.5% is observed when fibers of both cultivars reached 36 dpa. The decrease of the amount of adsorbed water from 10% to 5.5% when the fiber reaches 36 dpa could be due to a decrease in the specific surface per unit mass and to an increase in the structural organization of the cellulose macromolecules in the secondary cell wall (increased crystallinity).[25]

Weight loss between 150 and 450°C

Statistical analysis (analysis of variance) showed a significant effect of the development stage (dpa) on the percent weight loss in both regions (Table IV). The effect of the cultivar was found statistically

TABLE II
Image Analysis of Fiber Cross-Sections of TX19 and TX55 at 36, 50, and 61 dpa

	dpa	Perimeter (μm)	Wall thickness (μm)	Theta (no unit)
TX19	36	54.1	1.84	0.394
	50	52.1	2.94	0.591
	61	59.3	2.91	0.536
TX55	36	60.4	2.03	0.398
	50	61.2	2.85	0.512
	61	59.9	2.83	0.521

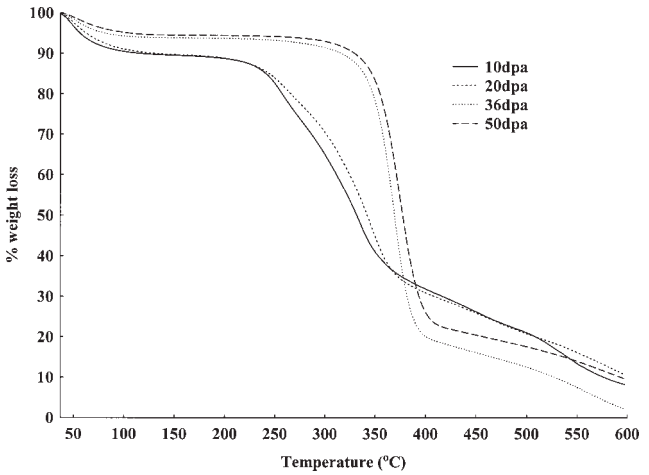

Figure 6 Percent weight loss of developing cotton fibers (TX55) at 10, 20, 36, 50 dpa.

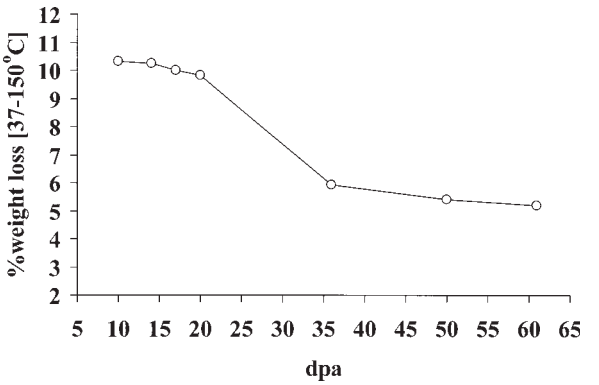

Figure 7 Thermogravimetric analysis: Percent weight loss between 37 and 150°C versus days postanthesis.

significant but the interaction cultivars × dpa was not. The average values of the percent weight loss for both TX19 and TX55 as function of days postanthesis are illustrated in Figure 8. There are no statistically significant differences between the percent weight loss of developing fibers at 10, 14, and 17 dpa. The percent weight loss averaged 59.78%. However, when the developing fibers reach 20 dpa, the percent weight loss averaged 69.73%. This increase could be attributed to an increase in cellulose deposition starting at or around 20 dpa. In addition to the decomposition of noncellulosic compounds, pyrolysis reactions of cellulose macromolecules also take place, confirming that synthesis of the secondary cell wall, which is composed of more than 99% cellulose, begins between 17 and 20 dpa. This hypothesis is supported by the results of Maltby et al.,[14] who reported that the abrupt increase in the percentage by weight of cellulose between 16 and 20 dpa defines the onset of secondary wall deposition. It is important to point out that in the study of Maltby et al., the noncellulosic polysaccharides were chemically extracted from the fibers. However, the FTIR and TGA data presented here are derived from raw unprocessed fiber. This simplified procedure eliminates lengthy extraction steps that may alter the chemical profile of fibers.

Evolution of the decomposition temperature in the region 325–425°C

First derivatives of thermograms of developing fibers were calculated to highlight the inflection points that indicate thermal transitions. As an illustration, Figure 9 shows the first derivatives of TGA of fibers at 10, 20, 36, and 50 dpa. The first derivative of fibers at 10 dpa shows thermal transitions at 214, 259, 300,

TABLE III
Variance Analysis: Effect of Developmental Stage (Day Postanthesis) of Cotton Fibers on the Percent Weight Loss in the Region 37–150°C

Parameter	df	*F*	Probability	% weight loss[a]
Intercept	1	17287.15	0.000001	
Variety	1	2.35	0.129949	
dpa (day post anthesis)	6	227.18	0.000001	
10				10.30[a]
14				10.25[a]
17				10.02[a]
20				9.82[a]
36				5.94[b]
50				5.39[c]
61				5.20[c]
Variety × dpa	6	2.16	0.057579	
Error	70			

df, degrees of freedom; *F*, variance ratio.
[a] Values not followed by the same letter are significantly different with α = 5% (according to Newman–Keuls tests).

TABLE IV
Variance Analysis: Effect of Developmental Stage (Day Postanthesis) of Cotton Fibers on the Percent Weight Loss in the Region 150–450°C

Parameter	df	*F*	Probability	% weight loss[a]
Intercept	1	36284.10	0.000001	
Variety	1	9.39	0.00309	
dpa (days post anthesis)	6	52.45	0.000001	
10				59.40[c]
14				59.06[c]
17				60.89[c]
20				69.73[b]
36				76.03[a]
50				70.84[b]
61				69.92[b]
Variety × dpa	6	1.62	0.153713	
Error	70			

df, degrees of freedom; *F*, variance ratio.
[a] Values not followed by the same letter are significantly different with α = 5% (according to Newman–Keuls tests).

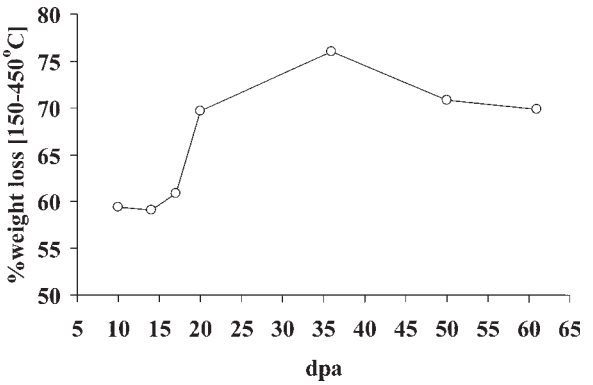

Figure 8 Thermogravimetric analysis: Percent weight loss between 150 and 450°C versus days postanthesis.

and 338°C. The first three transitions start disappearing at 20 dpa. These thermal transitions could be attributed to the decomposition of noncellulosic compounds and their disappearance at 20 dpa coincides with the decrease in the relative amount of noncellulosic compounds (decrease of the contribution of the primary cell wall, Fig. 2) and the formation of cellulose macromolecules.

Regarding the fourth transition, the statistical analysis (analysis of variance) shows significant effect of the stage of fiber development on the temperature of decomposition in the region of 325–450°C (Table V and Fig. 10). The temperature of decomposition increases with increasing developmental stage of the fibers. Fibers younger than 20 dpa are composed essentially of primary cell wall that contains between 35 and 50% cellulose (degree of polymerization 1000–3000[25]). Other primary cell wall components include noncellulosic materials, which have low decomposition points. As the secondary cell wall becomes increasingly thicker (fibers older than 20 dpa), cellulose becomes dominant (degree of polymerization > 14,000[25]) and more organized (degree of crystallinity > 60%), leading to an increase in the decomposition temperature.

TABLE V
Variance Analysis: Effect of Developmental Stage of Cotton Fibers (Day Postanthesis) on the Peak Temperature in the Region 325–450°C

Parameter	df	*F*	Probability	Peak temperature (°C)[a]
Intercept	1	454235.4	0.000001	
Variety	1	0.1	0.732378	
dpa (day post anthesis)	6	112.1	0.000001	
10				333.64[c]
14				335.32[c]
17				341.60[b]
20				344.71[b]
36				366.80[a]
50				365.13[a]
61				363.32[a]
Variety × dpa	6	11.1	0.000001	
Error	70			

df, degrees of freedom; *F*, variance ratio.
[a] Values not followed by the same letter are significantly different with α = 5% (according to Newman–Keuls tests).

CONCLUSIONS

Structural changes that occur at different developmental stages of cotton fibers beginning at 10 dpa were investigated using FTIR and TGA. FTIR results showed that vibration bands assigned to non-cellulosic compounds in FTIR spectra of fibers at 10, 14, 17, and 20 dpa are not detectable at 36 dpa and above. These results are consistent with the onset of the secondary cell wall synthesis around 21 dpa. Because the contribution of the primary cell wall to the total weight of the fiber decreases with increasing fiber maturity, the relative amount of noncellulosic compounds detected by FTIR decreases with

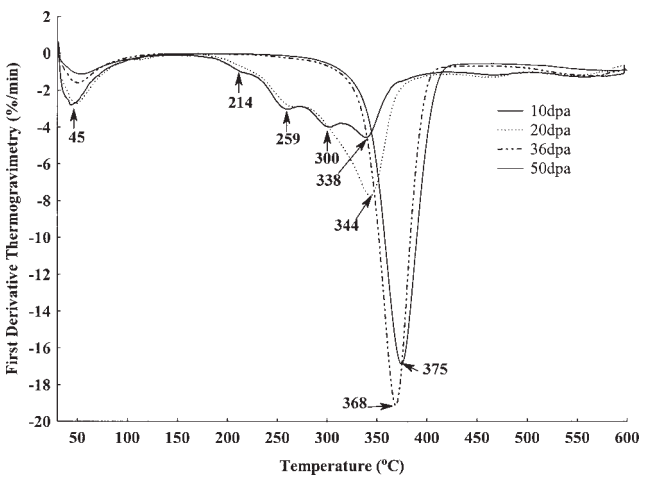

Figure 9 First derivative thermogravimetry of developing cotton fibers (TX55) at 10, 20, 36, and 50 dpa.

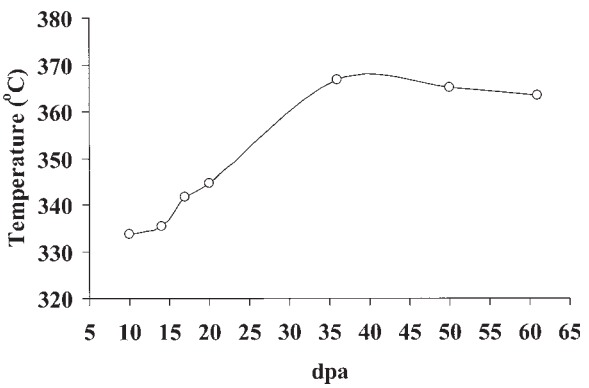

Figure 10 Thermogravimetric analysis: Evolution of the peak temperature as function of days postanthesis.

increasing dpa. TGA results suggest that the switch between primary cell wall and secondary cell wall deposition may begin shortly before 20 dpa (between 17 and 20 dpa). These results are in agreement with previous studies, where the cell wall components of developing fibers were extracted and analyzed using gel permeation chromatography. The advantages of using FTIR are that no extraction is needed, no preparation of the samples is needed, ease of analysis, and the testing is nondestructive allowing other analysis to be performed on the same set of samples such as TGA. Because of the potential of the FTIR as nondestructive analytical technique for cell wall components analysis, we continue developing this methodology. This could pave the way to develop a method for maturity analysis in developed cotton fibers. In addition, in our ongoing studies, we are planning to include fiber crystallinity measurements and correlate the data with TGA and FTIR measurements.

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