## DEVELOPMENT OF SECONDARY CELL WALL IN COTTON FIBERS AS EXAMINED WITH FT-IR SPECTROSCOPY M. Santiago Cintrón D. Hinchliffe Southern Regional Research Center, Agricultural Research Service, USDA New Orleans, Louisiana

#### Abstract

An examination of band development in Fourier transform-Infrared (FT-IR) spectra of developing cotton fibers (18 – 40 days post-anthesis or DPA) was performed. Two key spectral regions, the OH bending region, 1800-1500 cm<sup>-1</sup>, and the fingerprint region with multiple C-O vibrations, 1250-850 cm<sup>-1</sup>, displayed significant changes as the cotton fiber secondary cell wall developed. A transition in the shape and position of the OH bending band was observed between 24 and 28 DPA. An increase in the intensity of the C-O stretch bands at 1002 and 985 cm<sup>-1</sup> was also observed. Previous band assignments suggest that the C-O vibrations that undergo a significant intensity increase after secondary cell wall development arise from less frequent conformations of the cellulose alcohols. In contrast, bands from dominant C-O vibrations undergo a modest intensity increase during cell wall development.

#### **Introduction**

Cotton fibers are long, single cells that grow from the epidermal cells of the cotton seed. When mature, these fibers are mostly composed of cellulose, but they also contain small amounts of nitrogenous material, pectic substances, wax, sugar, organic acids and trace amounts of other chemical compounds (Kim and Triplett, 2001). Due to their length, and relative structural and biological simplicity, cotton fibers are particularly suitable for examining changes that occur during plant cell wall development. Molecular biology tools have been extensively employed in the examination of cotton plant cell wall development (Abidi et al., 2007). However, these reports have not closely looked at changes to the hydrogen bonding region as the secondary cell wall develops. In this study, we sought to expand the investigation of cotton cell wall development with FT-IR spectroscopy.

# **Methods**

One genetic line of cotton, MD90ne, was grown in 2009 under standard field conditions in New Orleans, LA. The development program of MD90ne was previously described by Meredith (2005). Cotton samples were harvested at 18, 20, 24, 28, 32, 36 and 40 days post-anthesis (DPA). Samples from each developmental stage were examined with FT-IR spectroscopy; Vertex 70 (Bruker Optics, Billerica, MA) equipped with an attenuated total reflectance (ATR) accessory (Pike Technologies, Madison, WI). A total of 256 scans were measured for each sample point.

## **Results**

Vibrational spectra of MD90ne exhibit significant band changes at different developmental days. Figure 1 shows FT-IR spectra of MD90ne at 18, 20, 24, 28, 32, 36 and 40 DPA in the OH bending region, 1800-1500 cm<sup>-1</sup>, and fingerprint region with multiple C-O vibrations, 1250-850 cm<sup>-1</sup>. The OH bending peaks display significant band shape changes from 18 DPA to 40 DPA. (Figure 1, left). A wide, midsized band is observed between 1700 and 1580 cm<sup>-1</sup>. The peaks in the region have been previously described as markers of water absorption. At the first three developmental stages (18-24 DPA) peaks can be observed at 1630 and 1641 cm<sup>-1</sup>. Beginning at 28 DPA a shift to a lower wavenumber, now centered at 1619 cm<sup>-1</sup>, and a broadening of the peaks is observed. This transition has been previously attributed to an increase in the crystallinity of cotton fibers, since it suggests a change in the way water is absorbed into the amorphous regions of the fiber. However, further investigation with other reference crystallinity measuring methods (i.e.; x-ray powder diffraction) should be performed.



Figure 1. FTIR spectra of MD90ne cotton fibers harvested at 18, 20, 24, 28, 32, 36 and 40 DPA. The OH bending region is shown to the left, while the C-O stretching region is show to the right.

The intensity of C-O vibrations in the fingerprint region changes as MD90ne develops. Mature MD90ne fibers exhibit 4 intense stretches at 1052, 1028, 1002 and 985 cm<sup>-1</sup> (Figure 1, right). The first three bands correspond to vibrations from cellulose alcohols: C-O vibrations of the C(3)-O(3)H secondary alcohol (reported at 1060 cm<sup>-1</sup>; observed at 1052 cm<sup>-1</sup> for the 40 DPA sample), and vibrations of primary and secondary confirmations of the  $C(6)H_2$ -O(6)H primary alcohol (reported at 1035 and 1000 cm<sup>-1</sup>, respectively; observed at 1028 and 1002 cm<sup>-1</sup> for the 40 DPA sample; Maréchal and Chanzy, 2000). The forth band observed, centered at 985 cm<sup>-1</sup> has been described as arising from the C-O stretches of alcohols involved in weak hydrogen bonding, likely from surface cellulose chains. While less-developed fibers (18-24 DPA) still exhibit the peaks at 1002 and 985 cm<sup>-1</sup>, their prominence is diminished. Figure 2 shows FT-IR spectra of MD90ne at 18 and 40 DPA that have been baseline-corrected, normalized, and without a shift in absorbance axis; the figure better displays the decreased importance of the peaks 1002 and 985 cm<sup>-1</sup> for the immature fiber (18 DPA). A transition for this spectral change is not observed since the identified peaks maintain their positions. Notably, the intensity of the 1052 and 985 peaks have been previously used to measure the maturity ratio of developed cotton fiber samples (Liu et al., 2011). However, the findings presented here suggest that a similar principle could be used to examine cotton fiber cell wall development. Curiously, the two C-O vibrations that undergo relatively small intensity changes during cell wall development (1052 and 1028 cm<sup>-1</sup> for the 40 DPA sample) arise from cellulose alcohols in their more predominant arrangements. In contrast, the bands at 1002 and 985 cm<sup>-1</sup> arise from cellulose alcohols in secondary or minor conformations (Maréchal and Chanzy, 2000).



Figure 2. The C-O stretching region of MD90ne cotton fibers harvested at 18 and 40 DPA as observed with FT-IR spectrometer.

#### Summary

This study sought to explore the cell wall development of a cultivar, MD90ne, with FT-IR spectroscopy. Vibrational spectra of the fibers exhibit significant band changes at different developmental days. Notable changes occur for the OH bending and C-O stretching bands. While a transition in the shape and position of the OH bending band is observed, the timing of the transition is likely dependent on cotton growing conditions and genetic line. Spectral changes for the C-O stretches are also observed in the form of a marked increase in the intensity of the peaks at 1002 and 985 cm<sup>-1</sup>. According to previous band assignments, the C-O vibrations that undergo a marked intensity increase after cell wall development arise from secondary conformations of the cellulose alcohols. In contrast, bands from dominant C-O vibrations undergo a modest intensity increase during cell wall development. While further testing with a larger variety of cotton cultivars is needed, our findings suggest that the development of a cotton cultivar can be followed with FT-IR spectroscopy in a manner similar to previous maturity ratio examinations of fully developed cotton fibers.

## **Disclaimer**

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# **References**

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