The *Journal of Cotton Science* 4:97-104 (2000)
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PHYSIOLOGY

Cotton Fiber Growth and Development 2. Changes in Cell Diameter and Wall Birefringence

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INTERPRETIVE SUMMARY

The mature cotton fiber used in textile processing is the end product of a series of developmental events in the life of this ovule epidermal cell. Thus, improvements in cotton fiber properties for textiles depend on changes in the growth and development of the fiber.

Fiber perimeter is thought to be established early in fiber development and remain constant. Manipulation of fiber perimeter has a potential to impact the length, micronaire, and strength of cotton fibers. The perimeter of the fiber is regulated by biological mechanisms that control the expansion characteristic of the cell wall and establish cell diameter.

The purpose of this investigation is to test whether the fiber diameter remains constant during development. We monitored the changes in fiber diameter that occur during the elongation and secondary-wall synthesis stages of fiber development. Four varieties of cotton (*Gossypium hirsutum*, MD51ne, DP50, and DP90; and *G. barbadense* L.) were examined at various stages of development.

These data are the first to describe, in detail, the changes that occur in fiber diameter (and perimeter) throughout growth and development. All varieties exhibited significant increases in diameter and, thus, perimeter during the first 30 d of development. These data indicate that perimeter is a dynamic fiber property that is established during a long period of development, rather than during a short stage early in development.

By 20 d of development, fibers exhibit increasing levels of wall birefringence (a measure of the amount of organized crystalline material). In the cotton fiber wall, the development of birefringence indicates the synthesis and deposition of the crystalline arrays of cellulose microfibrils in the secondary cell wall.

The overlap of increases in fiber diameter and secondary wall synthesis indicate that the secondary wall has the ability to expand. These observations are consistent with the fiber growing via a diffuse growth mechanism (that is, addition of new cell membrane and wall throughout the length of the cell) during the entire elongation stage. Understanding the dynamics and the biological mechanisms that control fiber growth is of benefit to breeders, plant physiologists, and molecular biologists interested in manipulating fiber development to increase economic value.

ABSTRACT

Cotton (*Gossypium hirsutum* L, and *G. barbadense* L.) fiber perimeter is an important textile trait that directly affects quality properties, such as fiber length, strength, and micronaire. The literature is contradictory regarding whether or not fiber diameter changes during development. Changes in maximum fiber diameter, fiber length, and cell wall birefringence were measured throughout development in four cotton genotypes (*G. hirsutum*, cultivars MD51ne, DP50, and DP90; and *G. barbadense*). All genotypes exhibited significant increases in fiber diameter during the first 30 d of fiber development. The *G. hirsutum* genotypes all had similar final diameters; whereas, *G. barbadense* had a significantly smaller final diameter. The *G. hirsutum*, cv. DP90, exhibited the smallest change in diameter (only 33%), compared with the other genotypes examined (MD51ne, 42%; DP50, 62.5%; and *G. barbadense*, 35%). All genotypes started secondary wall synthesis by 20 d post anthesis, as indicated by significant increases in wall birefringence. Simultaneous increases in fiber

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diameter and wall birefringence between 20 and 30 d post anthesis indicate that the secondary cell wall of cotton fibers is not rigid and is capable of expansion. These data indicate that fiber perimeter is a dynamic fiber property that changes significantly throughout a long period during development. This long window of opportunity during which fiber diameter and, thus, perimeter, can be modulated may provide a new avenue for changing fiber growth and development for the improvement in textile properties of cotton.

Improvements in fiber quality can take many different forms. Changes in length, strength, uniformity, and fineness are all needed to accommodate new techniques in textile spinning (Deussen, 1989, 1992; Zeronian, 1991). In one recent analysis, fiber perimeter was shown to be the single quantitative trait of the fiber that affects all other traits (Kloth, 1998). Fiber perimeter is the variable that has the greatest affect on fiber elongation and strength properties. While mature dead fibers have an elliptical morphology, living fibers have a cylindrical morphology during growth and development. Geometrically, perimeter is directly determined by diameter (perimeter = diameter $\times \pi$). Thus, fiber diameter is the only variable that directly affects perimeter. For this reason, understanding the biological mechanisms that regulate fiber diameter is important for the long-term improvement of cotton.

A review of the literature indicates that many researchers believe diameter is established at fiber initiation and is maintained throughout the duration of fiber development (DeLanghe, 1986). A few studies have examined, either directly or indirectly, changes in fiber diameter during development. Some studies indicate that diameter remains constant (Petkar et al., 1986); while others indicate that fiber diameter increases as the fiber develops (Boylston et al., 1990; Bradow et al., 1996; 1997). The studies that indicate changes in fiber perimeter either measured this trait indirectly (Bradow et al., 1996, 1997) or only measured limited developmental stages (Boylston et al., 1990). To date, no study detailing changes in fiber diameter during development has been published.

Cotton fiber progresses through four developmental stages (Jasdanwala et al., 1977): initiation, elongation, secondary wall synthesis, and maturation. While maturation occurs after boll opening and describes the drying of the mature, metabolically inactive fiber, the first three stages occur while the fiber is alive and actively growing. Fiber initiation involves the initial isodiametric expansion of the epidermal cell above the surface of the ovule. This stage may last only a day or so for each fiber. Because there are several waves of fiber initiation across the surface of the ovule (Stewart, 1975), one may find fiber initials at any time during the first 5 or 6 d post anthesis.

The elongation phase encompasses the major expansion growth phase of the fiber. Depending on genotype, this stage may last for several weeks post anthesis. During this stage of development the fiber deposits a thin, expandable primary cell wall composed of a variety of carbohydrate polymers (Meinert and Delmer, 1977). As the fiber approaches the end of elongation, the major phase of secondary wall synthesis starts. In cotton fiber, the secondary cell wall is composed almost exclusively of cellulose. During this stage, which lasts until the boll opens (50 to 60 d post anthesis), the cell wall becomes progressively thicker and the living protoplast decreases in volume. There is a significant overlap in the timing of the elongation and secondary wall synthesis stages. Thus, fibers are simultaneously elongating and depositing secondary cell wall.

The establishment of fiber diameter is a complex process that is governed, to a certain extent, by the overall mechanism by which fibers expand. The expansion of fiber cells is governed by the same related mechanisms occurring in other walled plant cells. Most cells exhibit diffuse cell growth, in which new wall and membrane materials are added throughout the surface area of the cell. Specialized, highly elongated cells, such as root hairs and pollen tubes, expand via tip synthesis where new wall and membrane materials are added only at a specific location that becomes the growing tip of the cell. While the growth mechanisms for cotton fiber have not been fully documented, recent evidence indicates that throughout the initiation and early elongation phases of development, cotton fiber expands primarily via diffuse growth (Tiwari and Wilkins, 1995; Seagull, 1995). Later in fiber development, late in cell elongation, and well into secondary cell wall synthesis (35 d post anthesis), the organization of cellular organelles is consistent with continued diffuse growth (Seagull et al., 1998). Many cells that

expand via diffuse growth exhibit increases in both cell length and diameter; but cells that exhibit tip synthesis do not exhibit increases in cell diameter (Steer and Steer, 1989). If cotton fiber expands by diffuse growth, then it is reasonable to suggest that cell diameter might increase during the cell elongation phase of development.

Cell expansion is also regulated by the extensibility of the cell wall. Regardless of whether cell expansion occurs via tip synthesis or diffuse growth, the wall in the region of expansion must yield to turgor pressure if the cell is to increase in size. For this reason, cell expansion most commonly occurs in cells that have only a primary cell wall (Cosgrove, 1997). Primary cell walls contain low levels of cellulose. Production of the more rigid secondary cell wall usually signals the cessation of cell expansion. Secondary cell wall formation is often indicated by the development of wall birefringence.

The current study presents several areas of interest. We carefully examined cotton fibers during the elongation and secondary wall synthesis phases of development. Analyses of fiber diameter and cell wall birefringence show that fiber diameter significantly increased as fibers grew and developed secondary cell walls. Both cotton species and all the genotypes tested exhibited similar increases in diameter; however, the specific rates of change differed. Fibers continued to increase in diameter during the secondary wall synthesis stage of development, indicating that the synthesis of secondary cell wall does not coincide with the cessation of cell expansion.

MATERIALS AND METHODS

Two species of cotton were examined, *G. hirsutum* and *G. barbadense*. Three *G. hirsutum* genotypes were examined: MD51ne, DP50, and DP90. All plants were grown between March and October in a glasshouse with day/night cycle of 16/8 h with day/night temperature of 30/25 °C. Supplemental lighting was supplied using four 1000-W sodium vapor lamps evenly placed in the greenhouse. Plants were grown in 20-L (5-gal) pots of ProMix™ potting soil. Plants were watered as needed and fertilized once a month with a 15-13-12.5 (15-30-15, oxide form) general-purpose fertilizer (Miracle-Gro).

Flowers were tagged on the day of anthesis, and bolls were harvested at the appropriate day post-anthesis (10, 20, 30, 40, or 50 d post anthesis). Bolls were opened and fibers quickly placed in fixative (25% acetic acid: 75% methanol) to ensure that fibers did not dry and collapse. Fiber samples from at least five ovules, from two to three bolls, were examined for each d post anthesis. For each age, ovules of similar size were harvested from the mid region of the boll, and fibers were removed from random regions of the ovule. Using light microscopy and a calibrated ocular micrometer, a total of 50 fibers for each d post anthesis were measured at a final magnification of 100X. Care was taken to ensure that no flattened fibers were measured because flattening the fiber would alter the measured fiber diameter.

Fibers exhibit a region of taper extending approximately 0.3 mm from the tip where diameter increases with increased distance from the fiber tip. Fiber diameter was measured in that region of the fiber beyond the taper. Measurements of diameter beyond the tapered region of a fiber exhibited no significant changes (data not shown).

Wall birefringence was measured using a polarizing light microscope with a 50X polarizing objective lens and crossed polarizer and analyzer lenses. Only single fibers in the field of view were measured. The fibers used to measure birefringence were not the same fibers used to measure diameter. Comparing diameter and wall birefringence in the same fiber showed no correlation between the two traits. Fibers with a narrower diameter may exhibit either a greater or lesser relative birefringence when compared with fibers with a larger diameter (data not shown).

Relative birefringence was measured using the exposure meter on an Olympus AD exposure control unit attached to the microscope. Greater birefringence is indicated by shorter exposure time. Care was taken to standardize all settings on the microscope to ensure that changes in exposure time reflected changes in wall birefringence. For convenience of data presentation and interpretation, exposure times were inverted so that increasing numbers represent increases in birefringence. For

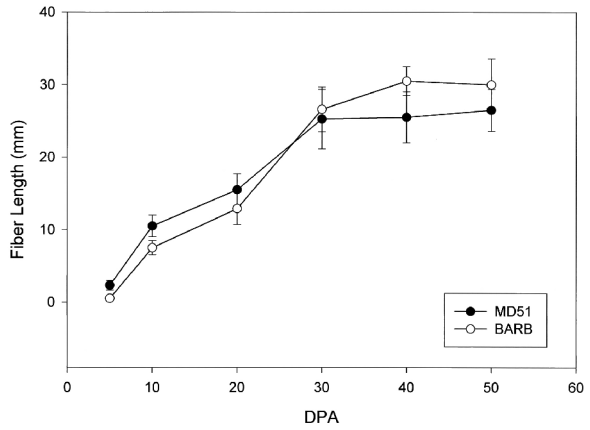

Fig. 1. Changes in length during fiber development in *G. hirsutum*, variety MD51 (MD51) and *G. barbadense* L. (BARB). (DPA, days post anthesis)

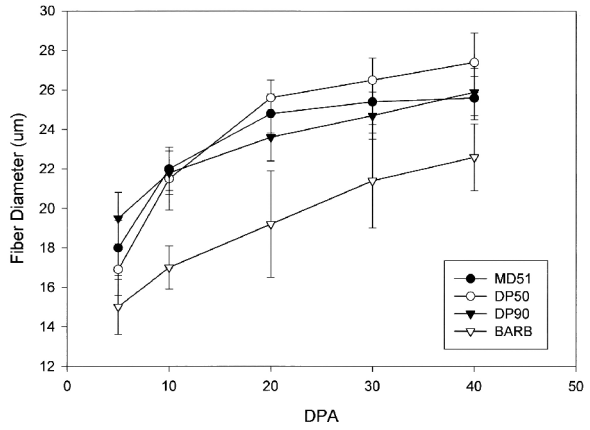

Fig. 2. Changes in fiber diameter during development in *G. hirsutum*, varieties MD51, DP50, DP90, and *G. barbadense* L. (BARB). (DPA, days post anthesis)

comparison, the value for the MD51ne, 40 d post anthesis fiber was arbitrarily set a 100%, and all other measurements compared with that value. This allows for comparison of developmental stages within a genotype and between different genotypes.

Total fiber length was measured by spreading fibers out from the ovule on a convex surface, using a stream of water. Fiber length was measured from the ovule to the outermost fiber tips, using a ruler. Ten measurements were taken from each fiber spread. To facilitate accurate measurements, ovules with attached fibers were gently boiled in 1.0 *M* HCl for 5 min, then washed with tap water to remove the acid (Gipson and Joham, 1969). Without acid treatment, fibers were tightly packed and coiled, but after treatment the stream of water easily straightened fibers.

For statistical analyses of all data, the Students *t*-test was used. Measurements were considered significantly different if $P < 0.01$.

OBSERVATIONS AND DISCUSSION

These studies were designed to determine whether fiber diameter remains constant during development, as suggested in the early literature (DeLanghe, 1986; Petkar et al., 1986), or changes during the growth and development of the fiber, as suggested by Boylston et al., 1990; and Bradow et al., 1996.

When increases in fiber length were monitored, both species of cotton exhibited similar elongation curves (Fig. 1). A rapid elongation phase occurred during the first 30 d post anthesis, after which elongation rate decreased, then stopped. All of the *G. hirsutum* genotypes exhibited similar patterns in fiber elongation (data not shown). Our fiber elongation data closely resembles previously published data (Naithani et al., 1982; Berlin, 1986). Fibers from the *G. barbadense* used in our study were somewhat shorter than previously reported field data (Hawkins, 1930), perhaps due to differences in the growth environment.

Accompanying the increase in length was an increase in fiber diameter (Fig. 2). All the cotton genotypes in our study exhibited significant increases in maximum diameter during the initial 30 d post anthesis. All genotypes and both species exhibited approximately a 30% increase in diameter between 5 and 30 d post anthesis, with DP50 exhibiting a 50% increase in diameter. All of the *G. hirsutum* genotypes had similar final fiber diameters; while that of the *G. barbadense* was significantly smaller.

These observations of final diameter generally agree with field data on fiber diameter among the different species and genotypes. However, it is interesting to note how the fibers of different species attain their final diameters. The initial diameter of the *G. barbadense* was significantly smaller than the diameters of the *G. hirsutum* genotypes. Of the latter, DP90 exhibited the smallest change in diameter (33%), compared with the other genotypes examined (MD51ne, 42%; DP50, 62.5%; and *G. barbadense*, 35%). The kinetics of the changes in diameter varied among the genotypes examined. For example, DP50 initially had a significantly smaller

fiber diameter than did MD51ne; and the diameter of DP50 remained smaller throughout the first 10 d of development. By 20 d post anthesis these two genotypes exhibited no significant difference in fiber diameter. The genotypic diameters remained the same throughout the 50 d of the study.

Differences in lateral expansion rates (changes in rates of diameter increase) may indicate differences among the cotton genotypes with respect to wall expansion characteristics, turgor pressure levels, or both. As diameter changes during a long period (30 d post anthesis), a long "window of opportunity" exists with the potential for modulating fiber diameter.

Our observations that cell diameter changes during fiber development added to our understanding of the mechanism of cell expansion in operation during cotton fiber growth. All plant cells expand via one of two mechanisms. Diffuse growth occurred when new wall and plasmalemma was added across the entire surface of the cell. Tip synthesis occurred when new wall and membrane are added at a specific location in the cell that becomes its growing tip.

Of the 20 or 30 different plant cell types found in plants, most exhibit lateral cell expansion, and these all expand via a diffuse growth mechanism (Green, 1969; 1980). Pollen tubes and root hairs expand via tip synthesis and exhibit no lateral cell expansion in sub-apical regions (Sievers and Schnepf, 1981; Steer and Steer, 1989). The initial stages of fiber elongation occur through a diffuse growth mechanism (Tiwari and Wilkins, 1995; Seagull, 1995). Later stages of fiber development have not been examined with respect to the mechanism of cell expansion. The lateral expansion of fibers during the first 30 d post anthesis (Fig. 2) is consistent with the functioning of a diffuse mechanism of cell expansion during that time period. These data support the possibility that cell expansion in cotton fibers occurs by a diffuse mechanism throughout the entire elongation phase.

Wall synthesis was monitored through the measurement of changes in form birefringence. Form birefringence is a measure of the amount of ordered material in an object, for example, the amount of ordered rods in a less refractive medium (Spencer, 1982). Cell walls have form birefringence due to the crystallinity of the cellulose microfibrils and their organization in a matrix of noncellulosic material. In

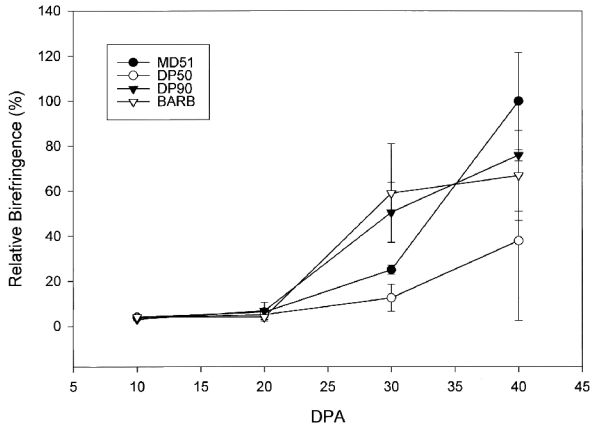

Fig. 3. Changes in wall birefringence during fiber development in *G. hirsutum* varieties MD51, DP50, DP90 and *G. barbadense* L. (BARB). (DPA, days post anthesis)

polarized light, birefringent objects appear bright on a dark background. As the amount of ordered cellulose in the fiber wall increases, the greater the form birefringence and the brighter the fiber appears. These data provide a measure of relative birefringence and can be used to monitor changes in the wall during development and to compare the different genotypes.

Prior to 20 d post anthesis fibers exhibit no detectable wall birefringence (Fig. 3). Fiber walls are not discernable from the dark background (data not shown). The lack of detectable wall birefringence coincides with the synthesis of primary cell wall (Seagull, 1986, 1993).

Between 20 and 40 d post anthesis fibers from all genotypes exhibited a dramatic increase in birefringence, indicating deposition of highly ordered arrays of cellulose microfibrils in the secondary cell wall. Both *G. barbadense* and *G. hirsutum* DP90 exhibited a rapid rate of wall development, as indicated by the relative slope of the birefringence curves (Fig. 3). Both DP50 and MD51ne exhibited similar and slower changes in wall birefringence with time (Fig. 3).

Comparison of fiber diameter with wall birefringence provides some apparent contradictions to what is commonly held as fact for cotton fibers. In all the genotypes examined, fiber diameter significantly increased between 20 and 30 d post anthesis (Fig. 2), a period when the cell wall exhibited significant increases in birefringence (Fig. 3). The observed increases in wall birefringence

coincide with higher rates of wall synthesis and dry weight accumulation in fibers (Ryser, 1985; Basra and Malik, 1984). Increases in fiber dry weight are the result of increases in the synthesis of cellulose (Meinert and Delmer, 1977). The cell wall that develops during this phase has been termed secondary cell wall and is composed almost exclusively of high DP (degree of polymerization) cellulose (Marx-Figini, 1982).

Unlike secondary cell walls of other plant cells, the cotton fiber secondary cell wall contains little noncellulosic component and no lignin (Meinert and Delmer, 1977). The secondary cell wall must be plastic enough to allow for the observed increases in fiber diameter. As lignin is one of the wall components thought to strengthen and make the wall rigid, the lack of lignin is consistent with the observed increase in fiber length and diameter. Many textbooks on plants define secondary cell walls as the wall deposited after cell expansion has ceased (Fosket, 1994; Raven et al., 1999). This definition should be re-evaluated in light of the data presented here. When secondary wall synthesis was well underway, the fibers continued to increase in length and diameter, indicating that the secondary cell wall is flexible enough to allow for the observed increase in size. The question remains as to whether the secondary walls of cotton fiber have similar or unique expansion properties when compared with the secondary cell walls of other mature plant cells.

The extent of cell expansion varied among the genotypes examined. Both MD51ne and DP90 exhibited no significant increases in diameter between 30 and 40 d post anthesis ($P = 0.3$ and 0.01, respectively). However, DP50 and *G. barbadense* did exhibit significant increases in diameter between 30 and 40 d post anthesis. The development of secondary cell wall does not appear to alter changes in fiber diameter since *G. barbadense* exhibited a significant increase in wall birefringence between 20 and 40 d post anthesis yet continued to increase in cell diameter (Figs. 2, 3). The data indicate that cessation of fiber elongation may not be correlated with cessation in diameter increase. For example, *G. barbadense* stopped elongating by 40 d post anthesis (Fig. 1), yet showed a significant increase in fiber diameter (Fig. 2). Detailed data on cell expansion during secondary wall synthesis in other plant cells is not readily available. Thus, it is difficult to determine if the cotton fiber secondary wall behaves like secondary walls in other plant cells. Regardless of whether or not the observations on cotton fiber secondary wall are applicable to other plant cell systems, an understanding of the cotton fiber system is essential to future efforts directed at modifying fiber growth.

From other plant cell systems, parameters such as the organization, composition, and thickness of the wall are all implicated in controlling wall extensibility. Wall thickness (as indicated by the level of birefringence) is not solely responsible for the regulation of fiber diameter. Fibers of *G. barbadense* exhibited a steady increase in diameter despite a large increase in wall birefringence (Figs. 2, 3). All four genotypes exhibited changes in the rate of diameter change (Fig. 2) between 5 and 20 d post anthesis, yet wall birefringence remains relatively constant during that time period (Fig. 3). Clearly, changes to the cell wall solely are not responsible for regulating fiber expansion.

The interplay of many processes, such as changes in wall extensibility, turgor pressure, and membrane synthesis, must be elucidated before a full picture of fiber expansion can be achieved. For example, the different expansion characteristics may not be due to differences in the cell wall but to differences in the turgor pressure exerted by the protoplast on the wall. Generation of turgor pressure is the mechanism that drives cell expansion in cotton fibers (Wilkins, 1992; Basra and Malik, 1983). The greater the turgor pressure, the more the cell wall is likely to yield and the greater the fiber expansion. Fiber turgor pressure is modified through changes in the osmotic potential of the cell (Wilkins, 1992; Kloth, 1992).

The concentration of malate and K^+ in the cytoplasm is thought to play a central role in generating the osmotic potential that generates turgor pressure (Dhindsa et al., 1975). Malate metabolism is regulated by the enzyme malate dehydrogenase. While evidence in the literature is somewhat contradictory, recent studies (Wafler and Meier, 1994; Ferguson et al., 1996) indicate that malate metabolism increases during early secondary wall synthesis. The resulting increase in turgor pressure may provide the driving force for the observed increase in fiber diameter. Data on the relative osmotic potentials of these genotypes might provide insight into the forces that drive fiber expansion.

We do not know how the cell regulates the relationship between increases in length and diameter. In both cases the cell wall must expand or stretch. However, the direction of expansion in each case is different. The patterning of microfibrils in the cell wall has been proposed to regulate wall expansion characteristics (Green, 1969, 1980; Seagull, 1994). Modulation of cellulose microfibril patterns in the developing cotton fiber may be a viable method for changing cell wall expansion properties and, thus, shifting expansion away from increases in diameter and toward further increases in length.

CONCLUSIONS

These data clearly illustrate that diameter (and thus perimeter and fineness) is not set during fiber initiation but is a dynamic trait that changes throughout fiber growth and development. As similar changes in diameter were observed in four genotypes from two species, it is reasonable that this trait might be common to most, if not all, cotton genotypes.

The fact that fiber diameter can be altered is not a new observation. It is well documented that the environment can alter final fiber diameter. For example, drought conditions result in short fibers with increased diameter. The data in this paper describe the kinetics of fiber expansion and demonstrate that diameter changes throughout fiber development. The biological mechanism(s) that regulate fiber diameter need to be explored to determine whether fiber growth can be altered to produce finer fibers.

These data expand our understanding of the growth and development of cotton fiber. While the biological mechanisms regulating the observed increases in fiber diameter remain undescribed, it is clear that understanding these processes can have a direct impact on the textile properties of cotton fiber. Fiber diameter is a dynamic property that changes during growth. If diameter change can be manipulated, then improvements in the textile trait of fiber fineness can be achieved.

ACKNOWLEDGMENT

Supported by Cotton Inc. Core Development Grant.

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