Effects of exogenous application of GA_3 on fiber morphology and secondary wall synthesis

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Abstract

This study investigates the effects of exogenously applied Gibberellic acid (GA_3) on the fiber length, diameter, secondary wall thickness, birefringence, and cellulose content, in the commercial variety *G. hirsutum* 'MD51'. Fibers were exposed to two different treatment regimes: first five-days after anthesis or every-other-day, from anthesis until harvest (40 d post-anthesis). Untreated fibers exhibit significant increases in all parameters over the study period. Fibers treated every-other-day with GA_3 (2.7 μM) exhibited significantly greater overall length, a thicker secondary cell wall, increased wall birefringence and a greater cellulose content. Fibers exposed for five days to GA_3 were significantly longer than control fibers, but not as long as fibers treated every-other-day. Fibers treated with five-day exposure exhibited thicker secondary cell walls with increased levels of birefringence and increased cellulose content. Neither treatment resulted in a significant change in fiber diameter, as compared to controls. These data indicate that GA_3 plays an important regulatory role throughout fiber development, influencing both cell expansion properties and secondary wall synthesis.

Key Words:

Cotton Fiber Development, Gibberellic acid, *Gossypium hirsutum*, Plant Growth Regulators, Cellulose synthesis

Introduction

There is an extensive literature that examines the relationship between plant hormones and cotton fiber growth and development (reviewed by Basra and Saha, 1999; Davidonis, 1999). Although there is little doubt that plant hormones play a critical role in the growth and development of cotton fibers, many questions remain. What are the precise roles of specific hormones? When are they required, at what concentrations, and in what proportion with other hormones? Data has been collected using fibers grown *in plantae* and in culture. Because of the differences in growth and development observed between plant-grown and culture-grown fibers (reviewed by Kim and Triplett, 2001) it is difficult to interpret the literature that relates plant hormones to fiber development using these two fiber-growth systems. Measurements of endogenous hormone levels are done from fibers grown *in plantae*, whereas experiments on the effects of exogenous hormone application are done with fibers grown in culture. Data from both *in plantae* experiments and experiments on fibers grown in culture indicate that Gibberellic acid 3 (GA_3) and indole-3-acetic acid (IAA) are essential for cotton fiber differentiation and elongation (Jasdanwala et al., 1977; Momtaz, 1998; Basra and Saha, 1999). This manuscript will specifically focus on GA_3.

Studies done *in plantae* indicate that endogenous GA_3 levels are higher during the initial period (1-5 d post-anthesis) of fiber elongation (Nayyar et al., 1989; Chen et al., 1996) and drop off as fibers enter secondary wall synthesis (after 20 d post-anthesis)(Nayyar et al, 1989). Yang et el (2001) report GA_3 levels remain relatively low and constant during fiber development. Quantitative estimates of GA_3 levels in fibers vary, with estimates at 2 d post-anthesis ranging from approximately 500 ng per gm fresh

weight (Nayyar et al. 1989), to 75 ng per gm fresh weight (Yang et al., 2001), to 6000 ng per gm fresh weight (Chen et al., 1996). Estimates of endogenous levels of GA_3 in fibers at 30 d post-anthesis are more consistent with both Yang et al (2001) and Nayyar et al. (1989) finding levels of approximately 50 ng per gm fresh weight.

The ovule culture system developed by Beasley and Ting has been extensively used to determine the importance of plant hormones in fiber growth and development. (Beasley and Ting, 1973, 1974; reviewed by Davidonis, 1999; Kim and Triplett, 2001). *In vito* studies indicate that the addition of GA_3 to the culture medium is specifically required for elongation and secondary wall development of culture-grown fibers (Delangue, 1986). In culture grown ovules, exogenous application of GA_3, kinetin or naphthalene acetic acid had no affect on secondary wall thickening, whereas application of abscisic acid increased both wall thickness and cellulose content (Yang, et al. 2001).

Until recently, little work has been reported on the effects of exogenous application of hormones on plant-grown fiber development. With fibers grown *in plantae*, exogenous application of either IAA or GA_3 induces significant increases in fiber length (Seagull et al., 2000b; Oliveri and Seagull, 2000, Gould, 2002). Only treatment with GA_3 induces a significant increase in wall birefringence (Seagull et al., 2000b; Oliveri and Seagull, 2000, Gould, 2002) and a change in reversal frequency (Gould and Seagull, 2002). The observed increases in wall birefringence were interpreted to indicate an increase in cell wall synthesis (Seagull et al, 2000b); however, no direct measure of wall synthesis or cellulose content was done.

Other GA_3-induced effects on the cell wall include increases in cellulose biosynthesis in pea internodes (Kördel and Kutschera, 2000). Increases in cellulose

synthesis were related to increased sucrose synthase activity. In cotton fiber, recent work by Yang et al (2001), reporting no GA_3-induced increase in wall synthesis in cultured fibers, appears to contradict earlier work indicating the ability of GA_3 to alter wall thickness or dry weights (Kosmidou-Dimitropoulou, 1976, reviewed by Davidonis 1999). Data reported by Seagull et al (2000b) from plant-grown fibers are consistent with a GA_3-induced increase in cellulose synthesis however they used measurement of birefringence to indirectly examine cellulose synthesis.

In addition to effects on cell wall synthesis, Gibberellic acid-induced increases in cell and organ elongation appear to be related to an ability to alter microtubule arrays (reviewed by Shibaoka, 1994; Baluska et al., 1999; Foster et al., 2003). GA_3 application alters microtubule arrays in several ways: through an up-regulation of tubulin gene activity (Mendu and Silflow, 1993; Yoshikawa et al., 2003); altering expression of microtubule associated proteins thereby altering microtubule patterns in the cell (Burk and Ye, 2002); by causing a predominance of transversely oriented microtubules and thus wall microfibrils; and/or by stabilizing microtubule arrays (Huang and Lloyd, 1999). Alterations in microtubule arrays result in a change in microfibril patterns in the wall that in turn alters cell expansion properties, leading to an increase in cell elongation. The re-organization of microtubules before enhanced cell elongation indicates that the hormone alters the microtubules, which in turn results in increased cell elongation. In cotton fiber, exogenous application of GA_3 results in increased length of fibers grown in culture (Davidonis, 1993). However, fiber elongation appears to be dependent more on IAA than GA_3 (Dhindsa, 1978). Preliminary data from exogenous application of GA_3 and IAA to cotton bolls developing *in plantae* (Seagull et al., 2000b) indicate that both GA_3 and IAA

can alter fiber development however, GA_3 appears to induce a greater change than IAA.

This study compares the effects of two different GA_3 treatments, five-day (5D) and every-other-day (EOD) on fiber length, diameter, wall thickness, birefringence, tapered tip length and cellulose content on *G. hirsutum* variety MD51 from 10 to 40 d post-anthesis.

Materials and Methods

Plant Growth

Gossypium hirsutum, variety MD51, cotton plants were grown in a 6x10 ft. growth chamber, illuminated with a 1000W sodium vapor lamp on a 16/8 hour, light/dark cycle at a temperature of 30/25° C. Plants were grown in 20 L pots of Pro-mix soilless potting mixture (Priemier Horticulture, Riviere du Loup, Quebec), watered when needed and fertilized once a month with Miracle-Gro™ (The Scotts Co., Marysville, OH.) general-purpose fertilizer (as per manufacturers instructions).

Hormone Treatment

Stock solutions of Gibberellic acid ($C_{19}H_{22}O_6$, Sigma Chemical Co., St.-Louis, MO), 1.0 mg ml^{-1}, in 0.2 percent ethanol (C_2H_5OH), were prepared and diluted with tap water to a final concentration of 1.0 mg L^{-1} (2.7 μM). Three drops of treatment solution (total volume of 120 uL) was applied directly onto each boll with a Pasteur pipette, beginning on the day of anthesis. When petals are present (zero through 3 d post-anthesis) hormone was applied to the location where the stamens and petals fuse. Once petals fall off, hormone solution was applied where the boll wall fuses with the bracts. Hormone was applied either daily until 5 d post-anthesis (GA_3 5D) or every-other-day until harvest (GA_3 EOD). Untreated fibers received no treatment. Tap water was not used because a previous study indicated no significant difference between water application on ovaries/bolls and no treatment (Seagull et. al, 2000a). Flowers were tagged on the day of anthesis and bolls were harvested at 10, 20, 30 or 40 d post-anthesis. Harvested bolls were quickly opened, and then seeds and fibers placed in a fixative solution of 1 % glutaraldehyde, 25% acetic acid and 75% methanol.

Fiber Analysis

Bolls were selected from branch nodes eleven through twenty, at the first or second fruiting position. Four ovules, chosen from the mid-region of the locule, from four to five different bolls, from four different plants were collected at 10, 20, 30 and 40 d post-anthesis for each treatment application. Samples of whole fibers, removed from the boll, were teased apart on a microscope slide containing a few drops of distilled water. Fifty fibers for each age and treatment type, selected from the mid-region of the ovule (seed) were analyzed for each parameter measured (tapered tip length, diameter, wall thickness and birefringence). Repeated measures ANOVA were employed to determine statistical significance within treatment groups over time followed by separate one way ANOVA with Post Hoc Tukey Tests within and between groups ($p<0.05$ was deemed significant).

Fiber Length

Using the technique of Gipson and Johan (1969), cotton seeds and fibers were boiled in 1.0 N HCL for 10 minutes and washed in water to straighten the fiber for length measurements. The acid boiled ovules were placed on a convex surface and a stream of water rinsed the fibers to spread them out. Measurements were done with a ruler, measuring from the ovule to the outermost tips of the fibers. Populations of fibers, rather than individual fibers were measured. Ten measurements were done from each of five ovules.

Fiber Diameter and Wall Thickness

Fiber diameter and wall thickness were measured with an aus Jena light microscope with a 50X objective lens and a calibrated ocular micrometer. For diameter

and wall thickness, regions of fibers well away from the tip were measured. Fifty undamaged fibers that had retained cylindrical appearance were measured for each DPA and treatment. The same region of the fiber was used to measure wall thickness and fiber diameter.

Wall Birefringence

Relative changes in wall birefringence were measured indirectly as previously described (Seagull et al., 2000a). In brief, as birefringence levels increase, fibers appear brighter on a dark background. When photographing such fibers, as birefringence levels increase, exposure times decrease. An Olympus AD exposure control unit (exposure meter) mounted on an aus Jena polarizing light microscope with a 50X polarizing objective lens was used to measure relative changes in wall birefringence. First, microscope settings (condenser, aperture, light intensity) were standardized to assure that exposure times indicated by the meter represented changes in wall birefringence only. Single fibers were oriented in the field of view to produce maximum brightness. Exposure time was recorded. Greater birefringence was indicated by shorter exposure times. For convenience of data presentation and interpretation, exposure times were inverted so that increasing numbers represent increases in birefringence. For comparison, the value of the 40 d post-anthesis control fibers was arbitrarily set at 100%, and all other measurements compared with that value. This allows for comparison of developmental stages within a treatment and between different treatments.

Cellulose Content

For each treatment, eight fiber samples were obtained from the mid-region of four

ovules from four different bolls from different plants 40 d post-anthesis. Care was taken in ensure that entire fibers were harvested. The number of fibers contained in each sample was counted and the specific weight of each sample determined. The weight of individual fibers was determined by dividing the weight of sample by the number of fibers in the sample.

Results and Discussion

Boll location and fiber characteristics

Data from field-grown cotton indicates that various boll and fiber properties are affected by fruiting position (branch location and fruiting position) on the plant. Both branch location and fruiting position appears to affect boll size; bolls size decreases with increases in either branch node or fruiting position number (Jenkins et al, 1990). Also, fiber properties appear to be affected by branch location (node) and fruiting position. Decreases in wall thickness and fiber circularity were observed when comparing fiber from bolls harvested from increasing branch number (i.e. node 14 compared to node 7) (Bradow et al, 1997).

Field-grown plants develop bolls over several months (reviewed by Oosterhuis, 2001), thus the development of those bolls and the fibers contained inside are likely to be affected by seasonal changes that occur over that time. Under field conditions, bolls that develop on higher branches and fruiting positions are subjected to different environmental conditions that most likely influence fiber development. Unlike these previous studies, plants in this study were grown in growth chambers and thus not subject to environmental and seasonal variations in growth conditions. Fiber properties (wall thickness and fiber diameter) were measured on bolls from different regions of the plant (Figure 1). Neither branch number nor fruiting position had a significant affect on fiber diameter or wall thickness. These data are consistent with the hypotheses that with field-grown cotton, differences in fiber properties due to boll location on the plant may be more directly related to differences in environmental conditions during boll development, rather than location on the plant.

Changes in Fiber Morphology (total length and diameter)

Cotton fibers undergo most of their elongation before 30 d post-anthesis (reviewed by Delangue, 1986; Seagull, 2001). Early literature indicates that elongation slowed or ceased at the beginning of the secondary wall synthesis phase of fiber development (Jasdanwala et al, 1977). Other analyses indicate that there is a significant overlap between the phases of fiber elongation and secondary wall synthesis (Meinert and Delmer, 1977). Accompanying the changes in fiber length are changes in fiber diameter. Although originally believed to remain constant (DeLanghe, 1986), more recent data indicate that diameter increases as fibers develop, both during primary and secondary wall deposition stages (Seagull, et al., 2000a). In the present study, significant increases in length and diameter occur in all treated and untreated fibers throughout the observation period, from 10 to 40 d post-anthesis (Figures 2 and 3, Tables 1 and 2). These data are consistent with previous analysis of fiber elongation that indicates fibers elongate over much of their development (reviewed by Delangue, 1986; Seagull et al., 2000a, Seagull, 2001), including well into secondary wall synthesis.

Fibers treated with GA_3 (either EOD or 5D) exhibited a significantly greater final fiber length than untreated fibers (Figure 2, Table 1). Longer exposure to GA_3 (EOD) results in the longest fibers (Table 1). Early in development (10 d post-anthesis) fibers exposed to either GA_3 treatment are significantly shorter, however this inhibition of fiber growth is temporary because by 30 d post-anthesis, fibers treated with GA_3 (either EOD or 5D) are significantly longer than control fibers (Figure 2, Table 1). These data indicate that exogenous application of GA_3 may have resulted in hormone levels that exceeded optimum values, resulting in a short-term inhibition of fiber elongation. The

literature provides contradictory data on endogenous levels of GA_3 (discussed in the introduction) and on changes in endogenous levels. Chen et al (1996) report GA_3 levels remain relatively constant (6000 ng per gm fresh weight) between 1 and 5 d post-anthesis, whereas Nayyar et al. (1989) report levels increasing from 50 to nearly 300 ng per gm fresh weight over the same developmental period. In our experiments, the exogenous application of 2.7 μM GA_3 on the surface of the ovary may have an effect on hormone levels in the developing ovule and fibers. Our observations that long-term exposure (GA_3 EOD) results in an initial inhibition, followed by stimulation of fiber elongation (Figure 2, Table 1) are consistent with the hypothesis that the fibers may respond differently, depending on developmental stage. High levels of GA_3 may be inhibitory early in fiber development and stimulatory in later stages. Alternatively, if there is a drop in GA_3 levels during fiber development, as suggested by Nayyar et al. (1989), then exogenous application of GA_3 may raise overall hormone levels to an inhibitory level early in development, thus inhibiting fiber elongation. Whereas when GA_3 levels drop later in development, exogenous application may promote elongation. Further work needs to be done to distinguish between these two hypotheses.

Increases in fiber diameter during secondary wall synthesis appear to be a unique property of cotton. In other systems it is generally accepted that the production of secondary cell wall coincides with cessation of cell expansion (Fosket, 1994). Fibers from all treatments exhibit significant increases in fiber diameter during the experimental period (Figure 3, Table 2). Control and GA_3 EOD-treated fibers exhibit significant increases in diameter throughout the entire experiment, whereas GA_3 5D-treated fibers exhibit an increase only between 10 and 20 d post-anthesis (Table 2). By 40 d post-

anthesis there are no significant differences in fiber diameter among the treatments. (Figure 3, Table 2). Our observations of fiber diameter at 10 d post-anthesis indicate that GA_3 treatment results in fibers with a significantly smaller diameter. Thus, GA_3 treatment appears to reduce or inhibit the lateral expansion of fibers early in their development. These observations are consistent with the inhibition of fiber elongation that is observed over the same time period. As seen with fiber length, this inhibition appears temporary because by 40 d post-anthesis GA_3-treated fibers have the same diameter as control fibers.

Fibers exposed to GA_3 were significantly longer (Table 1). Increasing the exposure to GA_3 from 5D to EOD resulted in further increases in fiber length (Figure 2). Neither treatment with GA_3 resulted in a significant alteration of final fiber diameter (Table 2). Together, these data indicate that GA_3-induced changes in fiber growth are directed specifically towards wall expansion in one direction (resulting in longer fibers) and not directed towards a general increase in wall extensibility that would result in increases in both length and diameter. These data do not directly distinguish whether the changes in fiber growth are due to GA_3-induced changes in cell wall expansion properties due to changes in XET (xyloglucan endotransglycosylase) activity (Allen et al., 2000) or microtubule patterns (reviewed by Shibaoka, 1994). Our data are consistent with the work of Allen et al. (2000) indicating increased XET activity resulting in longer fibers, with no change in fiber fineness (diameter).

Our data indicate that short-term exposure to elevated levels of GA_3 can have long term effects on fiber development. Significant increases in fiber length with GA_3 5D are not observed until 30 d post-anthesis, i.e. 25 days after the cessation of hormone

application. Further work needs to be done to determine whether hormone levels remain high long after the exogenous treatments have ceased or that initial treatments cause a chain of effects that result in subsequent increases in fiber elongation.

Changes in Secondary Wall Synthesis (Wall thickness, relative birefringence, cellulose content)

In most plant cell systems, the secondary cell wall is characterized as being generally thicker than the primary cell wall, containing a much higher percentage of crystalline cellulose, and containing a wall-hardening component, lignin (Fosket, 1994; Taiz and Zeiger, 2006). The secondary cell wall of cotton fiber generally fits these characteristics; it is thicker and contains a much higher percentage of cellulose (Meinert and Delmer, 1977). In fact, the secondary wall of cotton fiber contains one of the purest forms of cellulose that is naturally produced (Marx-Figini and Schulz, 1966; Delmer, 1999). However, unlike the secondary walls of most plant cells, the secondary wall of cotton fiber lacks lignin (Meinert and Delmer, 1977). Previous work on secondary cell walls of cotton fiber indicates that this poly-lamellate structure is composed of layers of cellulose microfibrils that are oriented in steeply pitched helices around the fiber protoplast. As the wall thickens, the pitch of the helix increases until the final layers of microfibrils are deposited with an orientation that is near parallel to the long axis of the fiber (Seagull, 1986; 1993). The crystalline nature of the cellulose, along with its arrangement into parallel arrays of microfibrils results in the production of a cell wall that is highly birefringent. Birefringence has been used as an indicator of the presence (Seagull, 1986) and amount of cellulose (Seagull et. al, 2000a) in the secondary wall of

fiber. The level of birefringence can be changed by either increases in the amount of crystalline wall material or by changes in the degree of order in the crystalline material (i.e. a constant amount of wall material with increased order will result in increased wall birefringence). Previously reported increases in wall birefringence (Seagull et al., 2000a, b) may be due to changes in either of these parameters or changes in both.

The present study extends previous observations by adding data on wall thickness (Figure 4 and Table 3) and fiber weight. Fibers from all treatments exhibit significant increases in wall thickness between 10 and 40 d post-anthesis (Figure 4, Table 3). By 40 d post-anthesis fibers from both GA_3 treatments exhibit significantly thicker (approximately 25%) cell walls than the control fibers. Fibers receiving the longest treatments with GA3 (EOD) have the thickest walls (Figure 4, Table 3). The analysis of relative birefringence (Figure 5, Table 4) shows similar patterns of increase, however relative birefringence increases to a greater extent than wall thickness (i.e. 60 – 70% increase in birefringence for EOD treated fibers, as compared to 25 % increase in wall thickness, Figure 5) Fibers treated for 5 days exhibit a smaller, yet still significant increase in wall birefringence, as compared to control treated fibers.

We studied cellulose content by calculating individual fiber weight. We believe this is a better approach for two reasons. First, previous work indicated that the bulk of the weight of cotton fibers is due to cellulose (Meinert and Delmer, 1977; Delmer, 1999), thus increases in weight are most likely attributable to increases in cellulose content. Second, in preliminary work for this project we measured cellulose content using the Updegraff technique (Updegraff, 1969) and found the data not very informative. This is because the Updegraff procedure requires that a standardized, specific weight of fiber be

used for each analysis. This standardized weight of cotton will contain more or fewer fibers, depending upon how much weight (cellulose) is in each fiber, thus masking data on changes in weight of individual fibers.

Our analysis of fiber weight determined the number of fibers that constituted a specific weight, thus allowing the calculation of average fiber weight. This analysis indicates that control fibers weigh 4.9 μg per fiber; whereas fibers treated with GA_3 5D weigh 4.7 μg per fiber. These values are not significantly different. Fibers treated with GA_3 EOD weigh 7.5 μg per fiber, exhibiting a significant increase in weight of approximately 50% compared to control fibers. Because the secondary cell wall is almost exclusively made of cellulose (Delmer, 1999), differences in fiber weights can be attributed to differences in cellulose content. All of the estimates of secondary wall synthesis (wall thickness, relative birefringence and fiber weight) are consistent in that increases in weight coincide with increases in wall thickness and relative birefringence, indicating that hormone treatment results in an increase in cellulose synthesis.

A comparison of the various cell wall traits (wall thickness, birefringence and fiber weight) among the different treatments of fibers begins to dissect the relative contributions of "amount of ordered material" and "degree of order in the material" to wall structure. Between 30 and 40 d post-anthesis, control fibers exhibit increases in wall thickness, with no significant increase in wall birefringence. One plausible explanation as to why birefringence does not increase is that the overall order of the microfibrils in the wall must be decreasing. Because birefringence levels are affected by the order of the microfibrils within the wall (i.e. the more parallel the microfibrils are to one another, the more birefringent the wall), the addition of cellulose micorfibrils with an orientation that

is different from previously deposited microfibrils may not increase the overall crystalline order of the wall and thus not increase birefringence. It is possible that although more cellulose is deposited between 30 and 40 d post-anthesis, as indicated by increases in wall thickness, the degree of crystalline order in the wall does not increase, thus there is no increase in birefringence.

$GA_3$5D and GA_3EOD-treated fibers exhibit significant increases in both wall thickness and birefringence between 30 and 40 d post-anthesis. Comparing the two treatments at 40 d post-anthesis, fibers treated with GA_3EOD exhibit significantly greater wall birefringence and wall thickness. Also, these fibers exhibit an approximate 60% greater weight (7.5 μg per fiber, as compared to 4.7 μg per fiber). These data are consistent with longer hormone treatment resulting in more cellulose being deposited, in arrays that are more highly ordered.

This study is the first to document the effects of exogenous hormone application on fibers produced on developing ovules within the ovary (*in plantae*). We examined the changes that occur over time in specific morphological (length and diameter) and cell wall (thickness, birefringence and weight) traits. Based on the results and discussion above, we propose that the exogenous application of GA_3 causes an increase in cellulose synthesis, resulting in changes in wall chemistry (increased cellulose production). In addition, hormone treatment induces changes in wall organization (microfibrils are deposited in more organized arrays leading to increased birefringence) that might be the result of alterations in cytoskeletal function. These changes result in altered cell expansion and morphology, with fibers being longer.

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Figure Legends

Figure 1: The effects of fruiting site (branch number and fruiting position) on fiber diameter and wall thickness. Fifty fibers were measured for each data point. Values are means ± standard deviations. Branch number and fruiting position designation – 11.1 indicates branch # 11, fruiting position #1; 11.2 indicates branch 11, fruiting position #2; etc.

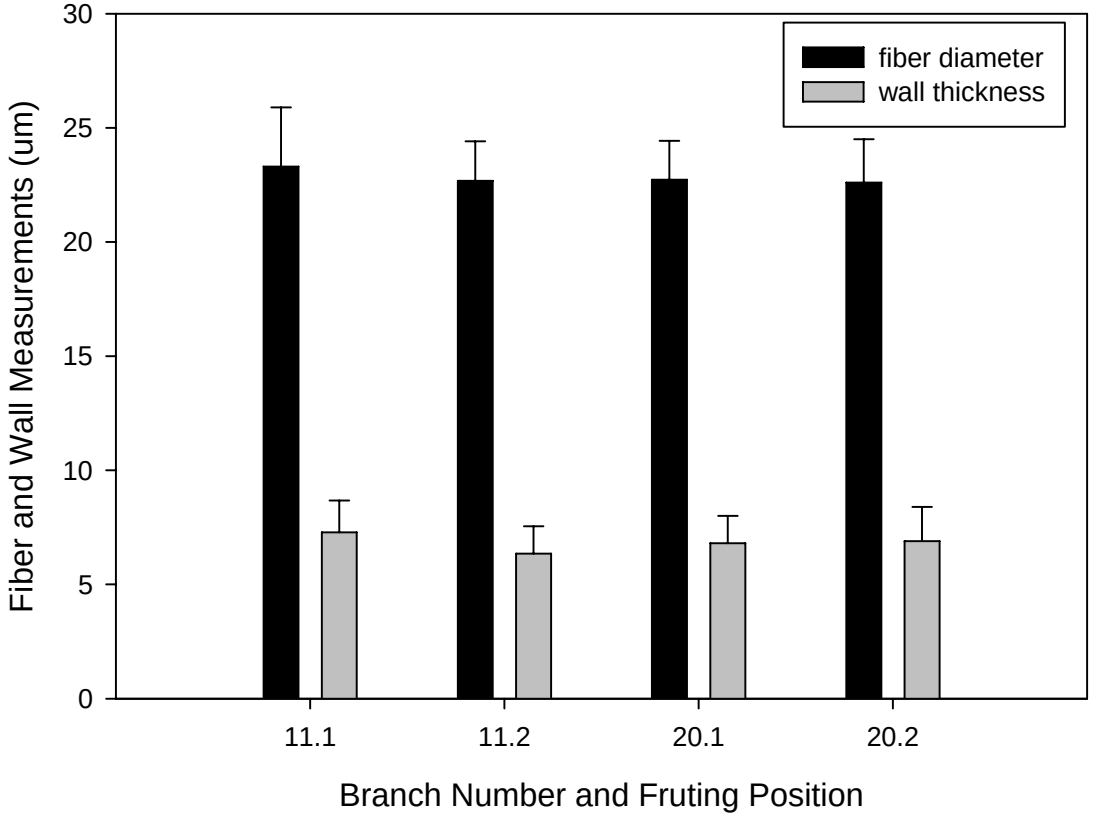
Figure 2: The effects of five day (5D) and every-other-day (EOD) treatment with GA_3 on fiber length. Fifty measurements were made for each data point. Values are means ± standard deviations.

Figure 3: The effects of five day (5D) and every-other-day (EOD) treatment with GA_3 on fiber diameter. Fifty fibers were measured for each data point. Values are means ± standard deviations.

Figure 4: The effects of five day (5D) and every-other-day (EOD) treatment with GA_3 on wall thickness. Fifty measurements were made for each data point. Values are means ± standard deviations.

Figure 5: The effects of five day (5D) and every-other-day (EOD) treatment with GA_3 on cell wall relative birefringence. Fifty measurements were made for each data point. Values are means ± standard deviations.

Figure 1



(My copy does not show the Y axis for the figures. It probably has something to do with my computer and how it reads the file. So I assume the axes are okay.)

Figure 2

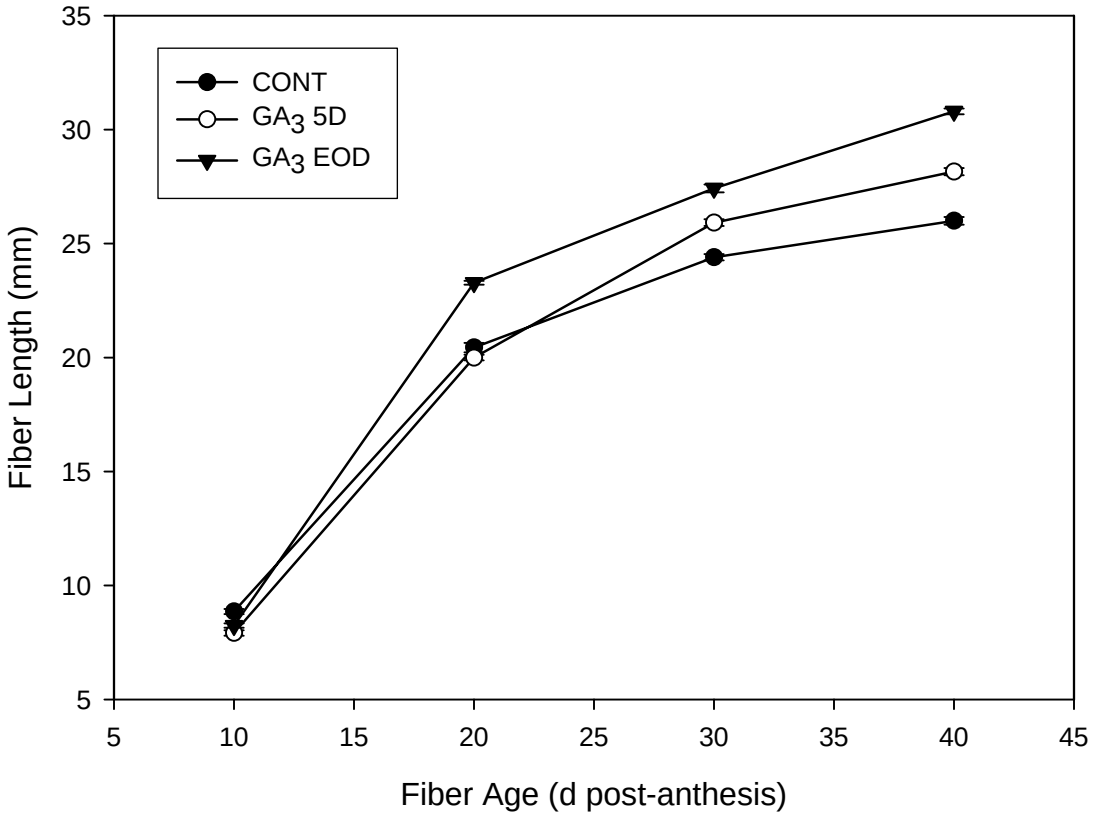

CONT
GA3 5D
GA3 EOD
Fiber Length (mm)
Fiber Age (d post-anthesis)
35
30
25
20
15
10
5
5
10
15
20
25
30
35
40
45

Figure 3

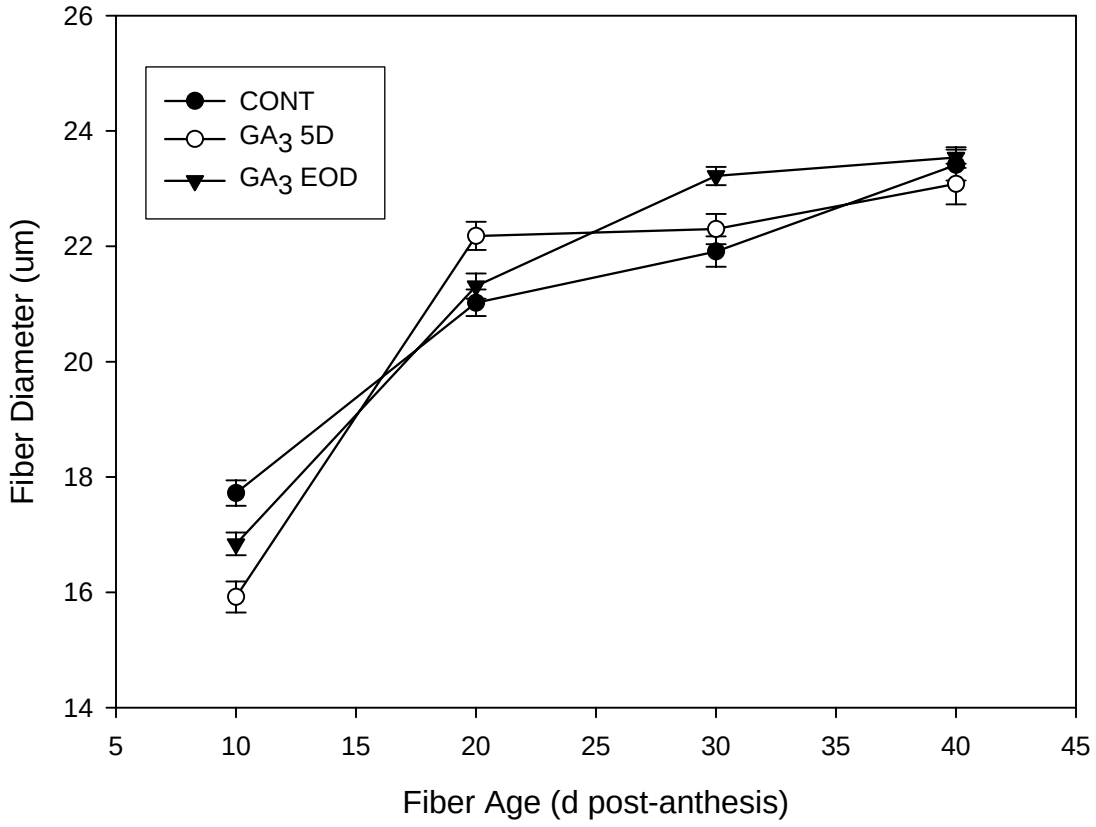

CONT
GA3 5D
GA3 EOD
Fiber Diameter (um)
Fiber Age (d post-anthesis)
26
24
22
20
18
16
14
5
10
15
20
25
30
35
40
45

Figure 4

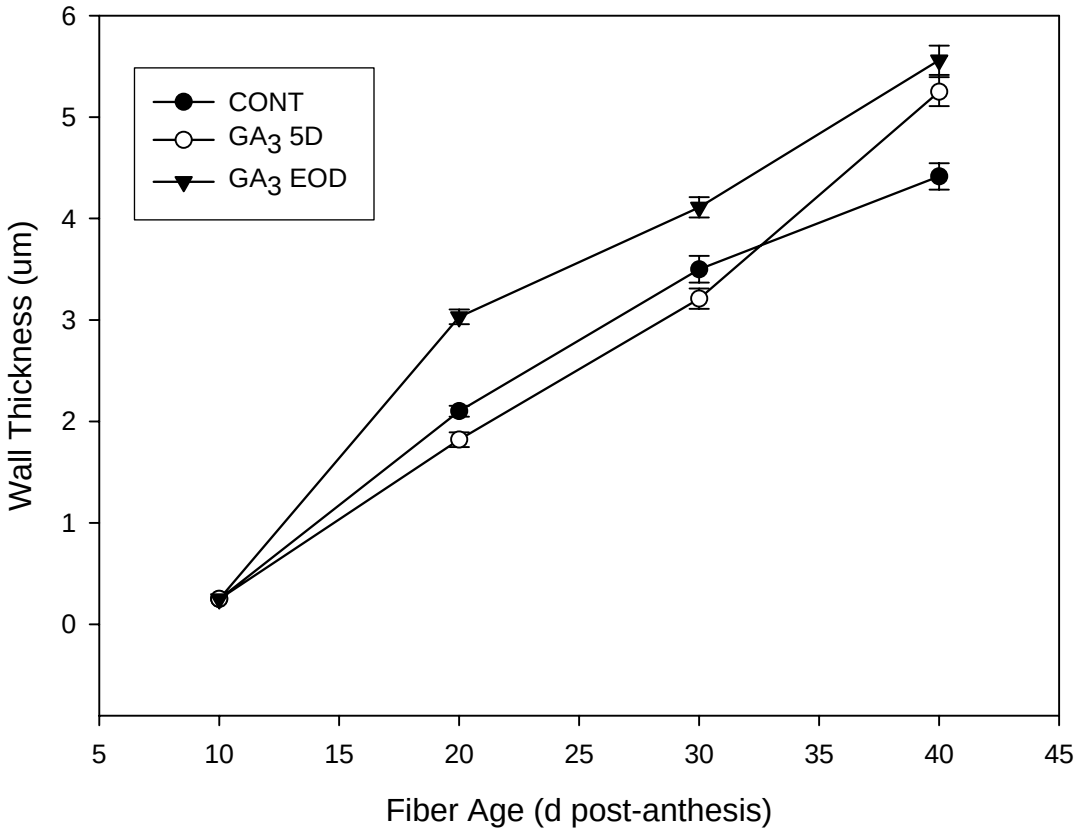

CONT
GA3 5D
GA3 EOD
Wall Thickness (um)
Fiber Age (d post-anthesis)
0
1
2
3
4
5
6
5
10
15
20
25
30
35
40
45

Figure 5

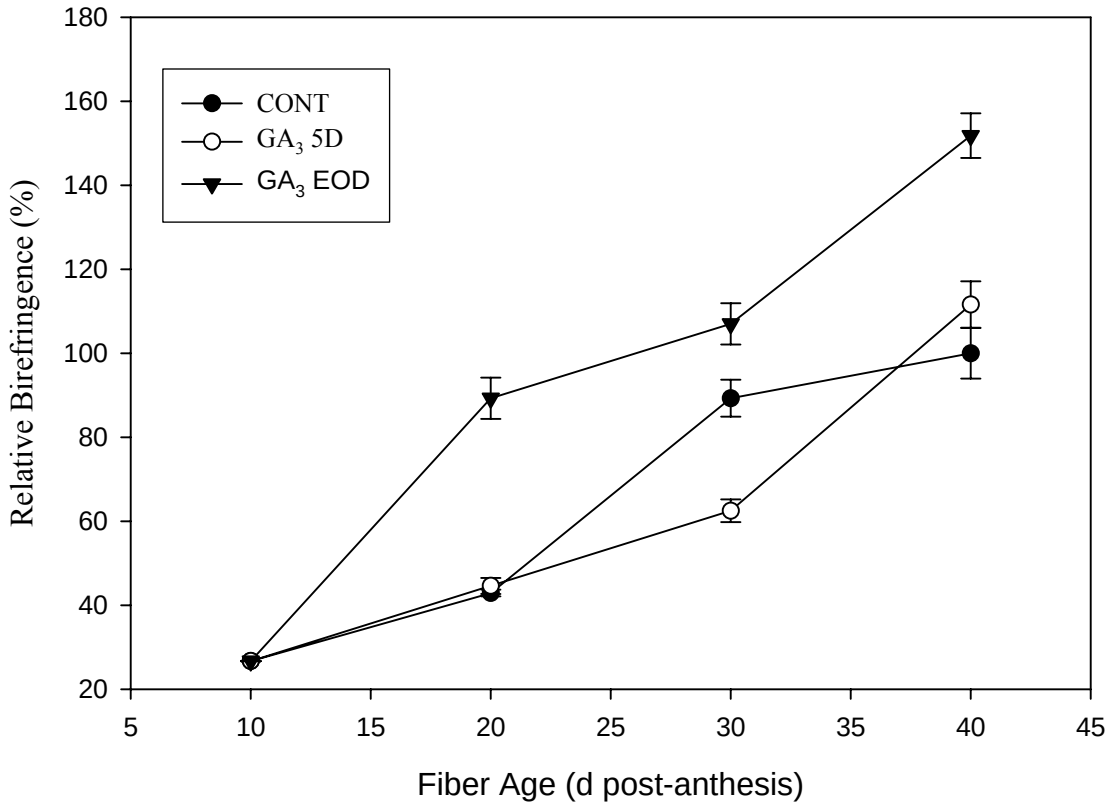

180
160
140
120
100
80
60
40
20
Relative Birefringence (%)
CONT
GA3 5D
GA3 EOD
5
10
15
20
25
30
35
40
45
Fiber Age (d post-anthesis)

Table 1: Statistical analysis of the effect of exogenous application of GA_3 on changes in fiber length using repeated measures ANOVA for within treatment groups over time followed by separate one way ANOVA with Post Hoc Tukey Tests within and between groups ($p<0.05$ was deemed significant).

d post-anthesis	Control	$GA_3$5D	GA_3EOD
10	a, A	a, B	a, B
20	b, A	b, A	b, B
30	c, A	c, B	c, C
40	d, A	d, B	d, C

Lower case letters represent comparisons within columns (age effects)
Upper case letters represent comparisons between columns (treatment effects)

Table 2: Statistical analysis of the effects of exogenous hormone application on changes in fiber diameter using repeated measures ANOVA for within treatment groups over time followed by separate one way ANOVA with Post Hoc Tukey Tests within and between groups ($p<0.05$ was deemed significant).

d post-anthesis	Control	GA_3 5D	GA_3 EOD
10	a, A	a, B	a, C
20	b, A	b, B	b, A
30	b, A	b, A	c, A
40	c, A	b, A	c, A

Lower case letters represent comparisons within columns (age effects)
Upper case letters represent comparisons between columns (treatment effects)

Table 3: Statistical analysis of the effects of exogenous application of GA_3 on changes in wall thickness using repeated measures ANOVA for within treatment groups over time followed by separate one way ANOVA with Post Hoc Tukey Tests within and between groups ($p<0.05$ was deemed significant).

d post-anthesis	Control	GA_3 5D	GA_3 EOD
10	a, A	a, A	a, A
20	b, A	b, B	b, C
30	c, A	c, A	c, B
40	d, A	d, B	d, C

Lower case letters represent comparisons within columns (age effects)
Upper case letters represent comparisons between columns (treatment effects)

Table 4: Statistical analysis of the effects of exogenous application of GA_3 on changes in relative wall birefringence using repeated measures ANOVA for within treatment groups over time followed by separate one way ANOVA with Post Hoc Tukey Tests within and between groups ($p<0.05$ was deemed significant).

d post-anthesis	Control	GA_3 5D	GA_3 EOD
10	a, A	a, A	a, A
20	b, A	b, B	b, C
30	c, A	c, B	c, C
40	c, A	d, B	d, C

Lower case letters represent comparisons within columns (age effects)
Upper case letters represent comparisons between columns (treatment effects)