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Cotton Fiber Development—Kinetics of Cell Elongation and Secondary Wall Thickening[1]

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ABSTRACT

The development of lint fibers of field-grown 'Stoneville 213' cotton (*Gossypium hirsutum* L.) was studied from anthesis to maturity with reference to lint fiber elongation, dry lint weight per seed, and dry lint weight per seed per unit of length. These variables were plotted against boll age and fitted to appropriate best-fit curves by computer curvilinear regression analysis. The curve-fitting procedures yielded mathematical equations which were differentiated to give accurate growth rate curves. The elongation rate curve showed that lint fiber elongation ceased at 27 days after anthesis. At that time, dry fiber weight and dry fiber weight per unit length had reached 40% of their final values. Splining analysis of the data shows that fiber dry weight per unit length ceased to be constant at 16 to 19 days after anthesis; theoretically, this is the age at which secondary wall thickening began. These data strongly suggests that the elongation and secondary wall thickening phases were not separated in time and that a substantial portion of secondary wall thickening occurred before fiber elongation ceased.

Additional index words: **Lint fiber elongation, Lint dry weight, Lint dry weight per unit of lint length, Cellulose.**

THE majority of cotton (*Gossypium hirsutum* L.) lint fibers are initiated from epidermal cells of the seed coat the day before to a day after anthesis (12). The growth of the fiber involves cell elongation and secondary wall thickening (11). Balls (2, 3) and Hawkins and Serviss (10) have stated that the initial period of fiber growth involves elongation, and secondary wall thickening does not begin until the cell elongation phase has been completed. Anderson and Kerr (1) reached similar conclusions in their classical study of cotton fiber development. Since these studies, it has been generally accepted that cotton fiber differentiation is characterized by an elongation phase followed by a secondary wall-thickening phase (6. 11, 16).

Benedict, Smith, and Kohel (4) have recently described cotton lint length and weight development. They found a substantial lint weight prior to the cessation of lint fiber elongation. In the present paper, we report a study of the kinetics of the growth and development of cotton fibers. Best-fit curves for the fiber growth parameters yielded mathematical equations which could be differentiated to give accurate growth rate curves. These rate curves (along with an electron photomicrograph of an elongating fiber) establish that secondary wall thickening of Phase II began well before the completion of fiber elongation Phase I.

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MATERIALS AND METHODS

'Stoneville 213' cotton was grown in the Cotton Variety Test Plots of the Texas A&M University Farm. There were six rows, each 1 m wide and 50 m long. An alley cut the plot in half, resulting in twelve 20 m rows. Four replications, each consisting of three rows, were designated. Early in the growing season, the stand was thinned to 1 m between plants to allow easier access to the plants for tagging and boll collection. Fertilization, irrigation, and pest control procedures were the same as those normally used in the test plot, and they were designed to maximize lint yield. Individual flowers were tagged daily for a 3-week period identifying their dates of anthesis. We attempted to collect data from bolls from flowers that had bloomed over as narrow a time span as possible to lessen the effects of environmental differences and position on the plant differences. Bolls were harvested at random within their respective replications at 3-day intervals.

A number of methods were tried for measuring lint fiber length. Combing was found to be difficult because of the mucilaginous matrix, which persists in the cotton boll throughout its development. The method adopted was the same as reported by Morris (15) and Gipson et al. (8, 9). The boll wall was carefully opened and the intact locules taken out of each cotton boll. The intact locules were dropped into boiling deionized water for 2 to 5 min to separate the seed (four drops of concd. HCl were added to 100 ml of water for bolls 20 days and older). The mass of seed cotton was broken up into its constituent seed and the lint hair straightened and floated out from each seed. The seed were transferred to a beaker of cool water. Ten seed from each boll were picked at random and the length of the lint hair was measured. Each seed was placed on the convex side of a watchglass, the lint hairs were flared out with a stream of water, and the lint length was measured from the rounded side of the seed adjacent to the chalaza end. The mean lint length was 2.70 cm (1.06 inch) for mature fibers. The mean fiber length for the ten seed was used as the value for each boll. The number of seed in each boll was counted and all seed transferred to 80% ethanol.

The lint was manually removed from the seed with a scalpel, without removing the coat or the dense mat of fuzz fibers near the seed coat. The lint was washed several times with 80% ethanol and dried in the oven for 2 days at 80 C before weighing. In order to remove the variability introduced into the lint weight per boll by the variable number of seed present, all weight data were expressed in terms of dry weight per seed. The values for weight of lint per seed per unit of lint length were also calculated.

Data for fiber length and fiber weight were analyzed by analysis of variance (randomized complete-block design). The standard deviations from the mean for each boll age were calculated for each of the three sets of data: length, weight, and fiber weight per unit length. Each set of data (plotted versus boll age) was fitted to an appropriate curve by computer curvilinear regression analysis. The fiber weight per unit length data were also fitted to three straight lines by a computer splining method. Rate curves were obtained by differentiating the best-fit equations. General references for the statistical procedures were Snedecor and Cochran (17) and Steel and Torrie (18).

Field-grown 'Dunn 56 C' (*G. hirsutum* L.) provided by Dr. J. R. Gipson, Texas A&M University, Texas Agricultural Experiment Station and Extension Center, Lubbock, Texas, was examined by electron microscopy. The fibers were removed and fixed while still in the field in cold 1.5% glutaraldehyde in 0.1 *M* cacodylate buffer at pH 7.2. The samples were then subjected to a phosphate-buffered osmium fixation (14), dehydrated with ethanol and embedded in Epon (13). The sections were stained with uranyl acetate and lead citrate containing Triton x-100 (5) and photographed in a Hitachi HU-11E electron microscope. The electron photomicrgoraph (Fig. 11) is a representative sample of at least 100 fibers and 10 different ovules examined at this boll age.

RESULTS

Analysis of variance revealed no significant differences among replications and highly significant ($P < 0.01$) differences among boll ages for both fiber length and fiber dry weight per seed.

When the data for lint length were plotted against boll age (the number of days after anthesis) as shown in Fig. 1, a hyperbolic pattern was indicated. The data were fitted to a polynomial. The regression equation is

$$y = a + bx + cx^2 + \ldots gx^6 + hx^7 \qquad [1]$$

where

$a = 1.09706$	$e = 0.558261 \times 10^{-3}$
$b = -0.703223$	$f = -0.136235 \times 10^{-4}$
$c = 0.153753$	$g = 0.174083 \times 10^{-6}$
$d = -0.127370 \times 10^{-1}$	$h = -0.905421 \times 10^{-9}$

The regression coefficient (r^2) is 0.982, and the multiple correlation coefficient 0.991. The best fit curve for lint length vs. boll age (Fig. 2) showed no appreciable lag time. Elongation continued for about 30 days with the measured lint length rather variable after the elongation period had ended. Hawkins and Serviss (10) and Morris (15) noted a sudden, temporary reduction in the rate of fiber elongation at different times in developing fibers. We cannot explain the reduction in fiber elongation. It may be due to a sudden temperature drop or to a short duration of drought. The differential curve of this polynomial (Fig. 3) indicated that fiber elongation stopped at 27 days after anthesis. The rate of lint length increase was greatest at 12 days.

Lint weight per seed data yields a more sigmiodal relationship when plotted against boll age. Figure 4 shows individual weight per seed observations vs boll age with ± one standard deviation from the means indicated. As the developmental period progressed, the data became more erratic, in general. The values for the final observation date, 48 days, are rather high, probably due to bias introduced in our sampling procedure. For example, at 48 days many bolls were open; we collected only unopened bolls, thereby selecting for bolls with a longer maturation period and possibly higher lint weight.

The lint weight per seed data were fitted to a logistic growth curve cited by Snedecor and Cochran (17):

$$W = A/(1 + BP^x) \qquad [2]$$

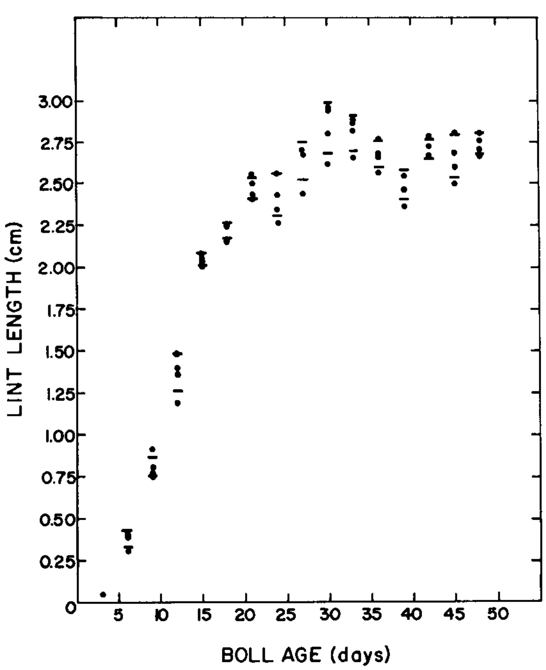

Fig. 1. Lint length vs boll age. The horizontal bars indicate ± one standard deviation (SD) from the mean value for that boll age.

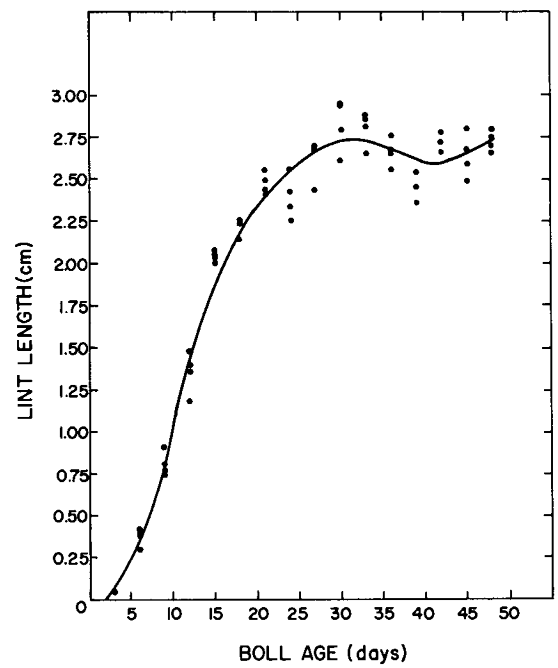

Fig. 2. Lint length vs. boll age with the best fit curve. The curve is defined by $Y = a + bx + cx^2 + \ldots hx^7$. The regression coefficient is 0.982, and the multiple correlation coefficient 0.991.

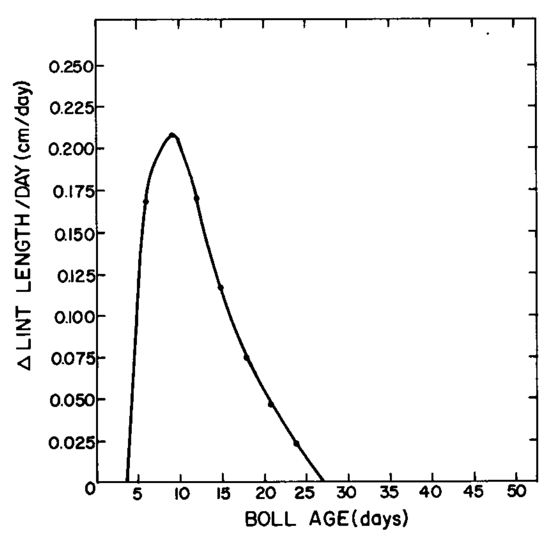

Fig. 3. The change in lint length per day vs. boll age. The curve is the derivative of the curve for Fig. 2. Fiber elongation ceases at 27 days of age.

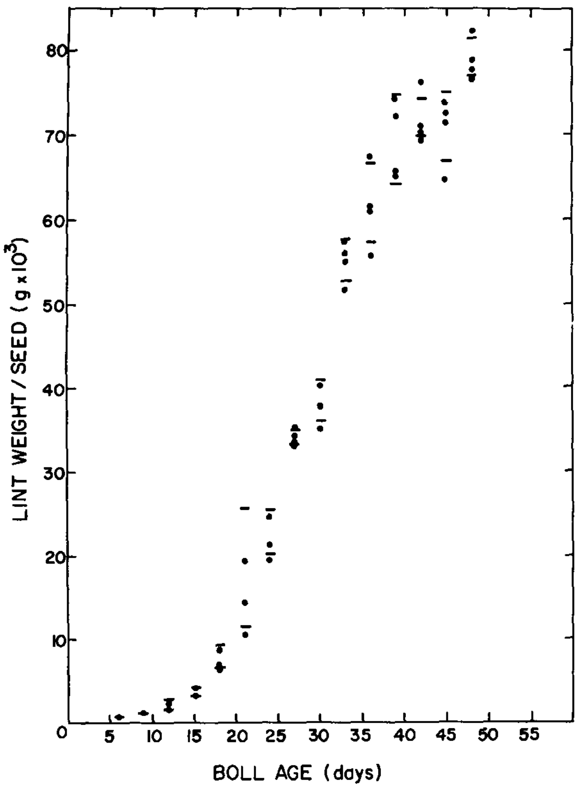

Fig. 4. Lint weight per seed vs. boll age. The horizontal bars indicate ± one standard deviation from the mean value for that date.

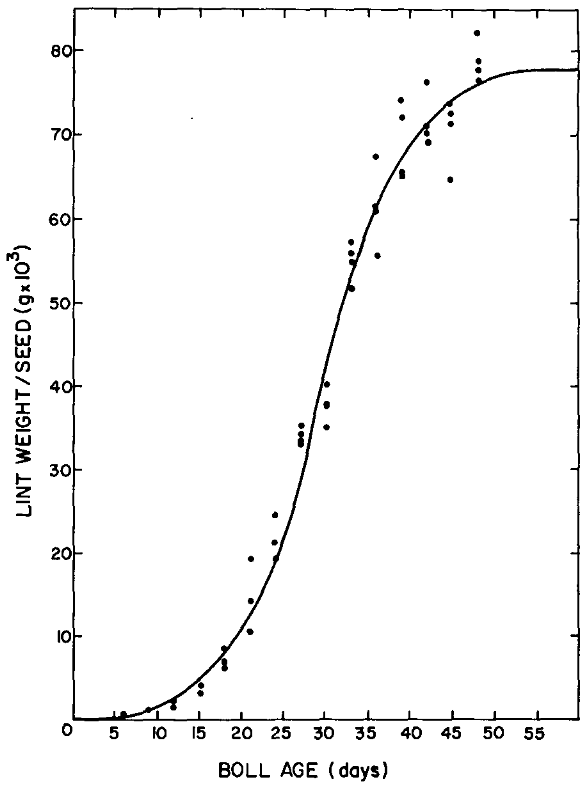

Fig. 5. Best fit curve for dry weight of lint fiber per seed vs. boll age. The curve is defined by equation [2]. The 95% confidence interval is ± 6.21 × 10^{-3} g per seed.

where

W = the dependent variable; lint weight per seed
x = the independent variable; boll age
A = the asymptote (as $X \rightarrow \infty$)
B = is related to the W-intercept as follows:

$$W_{x=0} = A/(1+B)$$

or

$$B = (A/W_{x=0}) - 1$$

P = the shape parameter, indicating the slope of the central portion of the curve (7).

The resulting curve is shown in Figure 5, in which

$A = 7.77811 \times 10^{-2}$
$B = 2.73864 \times 10^{2}$
$P = 8.23958 \times 10^{-1}$

Lint weight development showed a definite lag early in the period, but had increased greatly by 27 days after anthesis. The weight continued to increase (more slowly late in the period) throughout the measurement period, which ended at 48 days after anthesis. The curve past 48 days is an extrapolation. The 95% confidence interval for this curve is ± 6.21 × 10^{-3} g per seed.

The rate of lint weight increase (Fig. 6) was determined using the derivative of equation 2:

$$dW/dx = -[ABP^x (\ln P)/(1 + BP^x)^2] \quad [3]$$

This curve indicates that the maximum rate of dry lint weight increase occurred at 27 to 30 days after anthesis or about the time elongation ceased. After this point, the rate decreased until maturity.

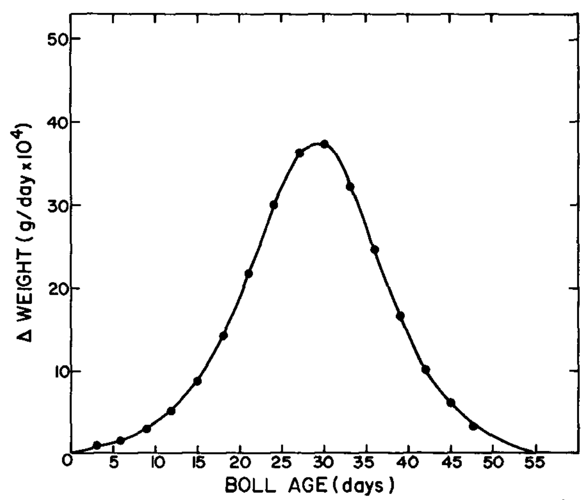

Fig. 6. The rate curve for lint weight increase. This is the curve of the derivative of Fig. 5, defined by equation [3].

By plotting the lint length and weight curves together (Fig. 7), we find that at 27 days after anthesis 40% of the final dry lint weight per seed had been accumulated.

The calculated values of lint weight per seed per unit of lint length were also fitted to the logistic growth curve described by equation [2] and used in characterizing lint weight development (Fig. 8). The coefficients found were:

$A = 2.985971 \times 10^{-2}$
$B = 1.74628 \times 10^{2}$
$P = 8.383476 \times 10^{-1}$

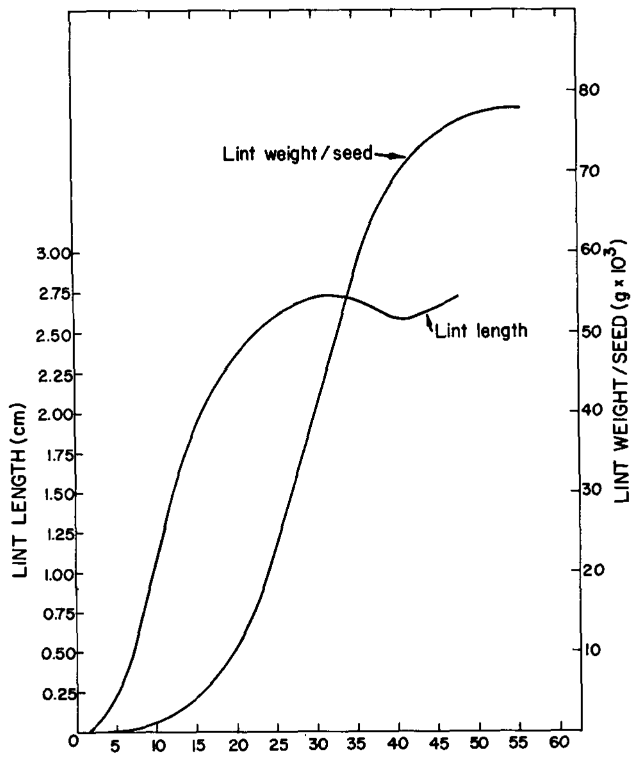

Fig. 7. Comparison of the developmental curves for lint length and lint weight per seed. At 27 days boll age, elongation ceased and lint weight per seed had reached 40% of its final value.

This curve is very similar to that for lint weight development, having an initial lag phase followed by a rapid increase which falls off toward the end of the measurement period. Again, the curve past 48 days is an extrapolation. At 27 days of boll age, 40% of the final lint weight per seed per unit of length had been attained. The 95% confidence interval for this curve is $\pm$ 30 $\times$ 10^{-4} g per seed/cm.

The rate curve for the lint weight per unit length data (Fig. 9) shows the greatest rate of increase at 30 days, just after the cessation of fiber elongation.

By fitting three straight lines to the data for the lint weight per unit length, a very graphic illustration of fiber cross-sectional development was obtained (Fig. 10). Initially, the ratio was fairly constant, as one would expect if no thickening and only elongation were occurring. This is indicated by the first line. Then the phase of rapid increase in lint weight per unit length began, as indicated by the second line. The first and second lines intersect at 17 days after anthesis; this is the age at which the walls began to thicken as well as to grow longitudinally. (The exact cut is at 17.51 days with a 95% confidence interval of $\pm$ 1.67 days.) After 40 days of boll age, the ratio value began to level off. The 95% confidence interval for this splined curve is $\pm$ 28.5 $\times$ 10^{-4} g per seed/cm.

When the cross-sections of fibers from a 19-day-old boll from 'Dunn 57C', field grown at Lubbock, Texas, were examined in the electron microscope, a substantial amount of secondary wall was found (Fig. 11). As one moves inward from the outer boundary of the

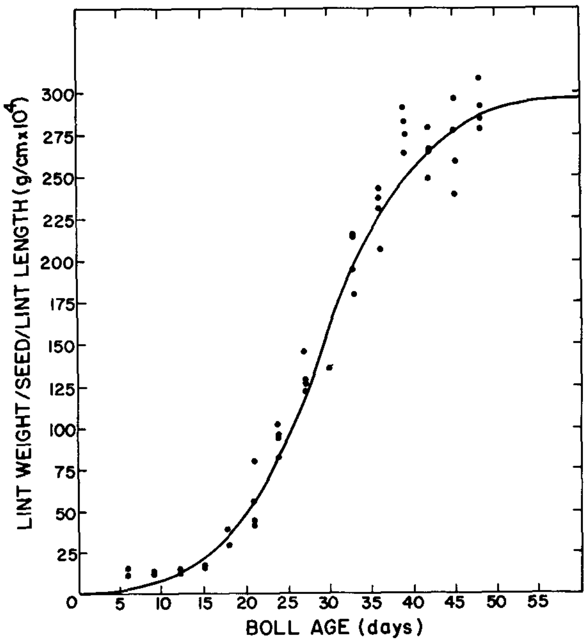

Fig. 8. Data for dry lint weight per seed per unit of lint length vs. boll age. The best fit curve is defined by equation [2]. The 95% confidence interval is $\pm$ 30 $\times$ 10^{-4} g per seed/cm. At 27 days, 40% of the final value has been attained.

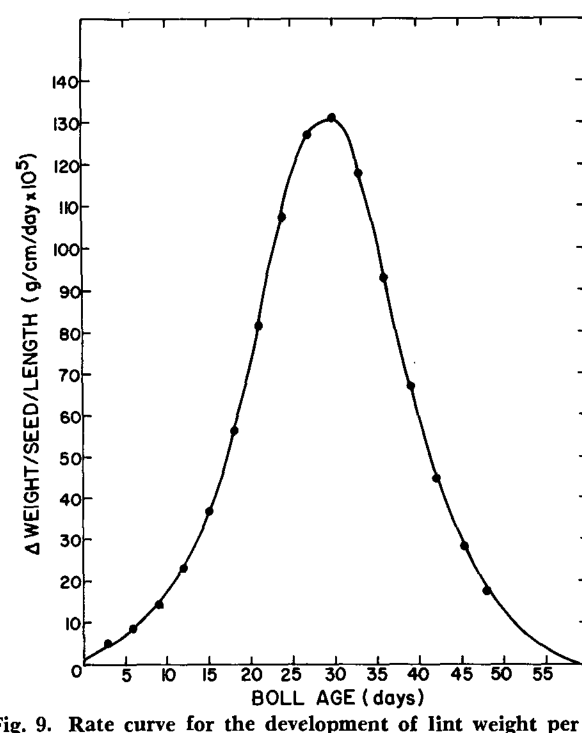

Fig. 9. Rate curve for the development of lint weight per seed per unit length. This is the derivative curve of Fig. 8, defined by equation [3].

cross-sections, one sees the primary cell wall (the thin, dark outer line), the relatively thick secondary cell wall, a thin layer of protoplasm, and the large central vacuole. Even at this early point in development, the preponderance of secondary wall material over primary wall material is evident. At this boll age, the very presence of a recognizable secondary wall is

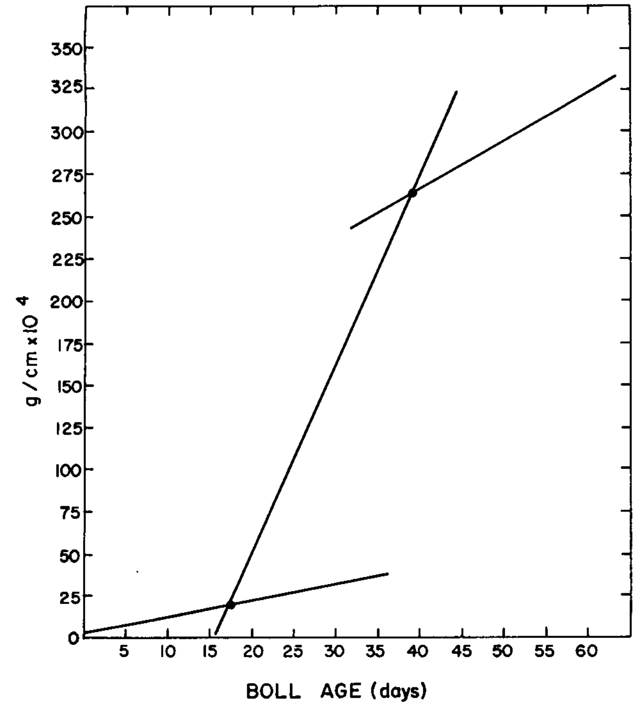

Fig. 10. Splined curve for lint weight per unit length vs. boll age. Note that the ratios cease to be constant at 17.5 days. The 95% confidence interval for this set of curves is $\pm$ 28.5 $\times 10^{-4}$ g per seed/cm.

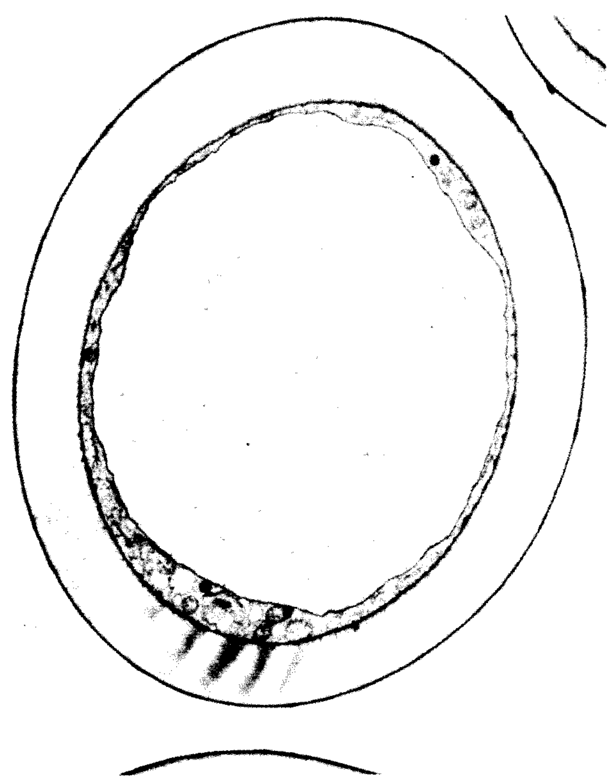

Fig. 11. Electron micrograph of the crosssection of a 19-day-old lint fiber from 'Dunn 57C.' The outer, dark band is the primary wall; the broader, light area, the secondary wall; the next thin layer, the protoplasm; and the central portion, the vacuole. Note the presence of the well-defined secondary wall at this young stage in lint development. Magnification 10,000 $\times$.

noteworthy in light of past theories of development. Although 19 days of boll development at Lubbock cannot be equated to 19 days of boll development at College Station, judged from the published values for the duration of elongation of several cotton varieties grown at the same location in Lubbock (8, 9), it should be noted that the thin section in Fig. 11 was taken from a fiber well within its elongation period.

DISCUSSION

The results in this paper show that a substantial amount of secondary wall was deposited in developing cotton fibers before the completion of fiber elongation. Differentiating the best fit curve for increases in fiber length showed that fiber elongation stopped at 27 days after anthesis. The data for lint weight increase showed that a maximum rate of dry weight deposition occurred at 27 days after anthesis. Inspection of the lint length and lint weight curves show that 40% of the final dry weight per seed had been reached at 27 days after anthesis. The splined curve for the lint weight per unit fiber length indicates that fiber wall thickness remained fairly constant until 16 to 19 days after anthesis and at this time the fiber began to thicken rapidly. It is clear that fiber thickness began 16 to 19 days after anthesis and that the rates of both lint weight per seed and weight per unit length increases were at or near their maximum values when fiber elongation ceased. These two observations indicate a considerable overlap in the elongation and secondary wall thickening phases in developing cotton fibers. The electron photomicrograph of the crosssection of a developing cotton fiber adds to the analysis of the weight and length parameters of developing fibers. The photomicrograph shows the relative amounts of primary and secondary wall deposited in a 19-day-old fiber. The thin section, was taken from a fiber well within its elongation period at Lubbock, and graphically illustrates the overlap in the elongation and secondary wall thickening phases in developing cotton fibers.

Our conclusions differ from the early developmental work on cotton fibers (1, 2, 3, 6, 10, 11, 16). In this view it might be important to review the 1930 paper by Hawkins and Serviss (10). This paper includes rate curves for fiber elongation of two cotton varieties, Pima and Acala and also contains fiber thickening analysis measured from the crosssections of embedded fibers by light microscopy. Their fiber elongation rate curves show that Pima and Acala fibers stop elongation 30 and 24 days after anthesis, respectively. The fiber thickening data show that Pima and Acala fibers have completed 60% and 25% of their secondary wall, respectively, at this time. These results of fiber length measurements and light microscopy of secondary wall thickening agree with the results in our present paper. Yet, these authors also conclude that no secondary wall thickening in cotton fibers occur until the completion of fiber elongation. A note of caution: our study has been concerned with the mathematical derivation of the kinetics of fiber length and weight increases in 'Stoneville 213'. The measurements of fiber length and weight primarily deal with the mean fiber length and mean fiber weight. There is no apparent method to study the amount of variation in fiber length and weight between the individual fibers of the population of fibers on an individual seed. In this respect, it is conceivable that some short fibers may complete elongation and begin secondary wall thickenings after 16 to 19 days after anthesis. The secondary wall formation in these fibers, which matured 50% earlier than the mean of the fibers of the seed, would contribute weight to the fibers prior to 27 days after anthesis when our results show fiber elongation has been completed.

Our data strongly suggest that, at least in 'Stoneville 213' under our specified growing conditions, the elongation and cell wall thickening phases occurred simultaneously for 10 days of a 48-day fiber developmental period. Fiber elongation rates, duration of elongation period, and rate of secondary wall deposition have been shown to vary among varieties and environmental conditions (8, 9). The degree of overlap of the elongation and secondary wall thickening phases has been shown to vary among varieties with different fiber fineness values (4). In this latter case, this knowledge may afford cotton breeders another means of tailoring a variety to a particular location. From a botanical viewpoint, the establishment of an overlap in the primary and secondary wall development suggests the need to examine the mechanism for coordinating these two processes simultaneously in a developing cotton fiber.

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