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Incorporation of ^{14}C-Photosynthate into Developing Cotton Bolls, *Gossypium hirsutum* L.[1]

C. R. Benedict, R. H. Smith, and R. J. Kohel[2]

ABSTRACT

Incorporation of ^{14}C-photosynthate into developing cotton (*Gossypium hirsutum* L.) bolls of several ages was studied in the cultivar 'Paymaster III.' Maximum incorporation of ^{14}C-assimilate into developing boll walls and fiber occurred at 8 to 10 and 23 to 40 days after anthesis, respectively. ^{14}C-photosynthate incorporation was rapidly increasing in the developing fibers prior to the completion of fiber elongation. For Paymaster III, 'Acala SJ-1,' and 'Stoneville 213' cultivars, the rate of increase of fiber dry weight was maximal prior to the completion of fiber elongation.

Additional index words: **^{14}C-photosynthate, Fiber elongation, Fiber dry weight increase, Cellulose.**

BROWN (1968) established, by the use of autoradiography, that a developing cotton (*Gossypium hirsutum* L.) boll receives ^{14}C-assimilate from its associated bracts, boll walls, subtending leaf, leaf subtending the sympodium, and leaves higher up on the same side of the main stem. No determinations were made as to the relative amount of carbon translocated from these different sources to the bolls at times of rapid leaf expansion or leaf senescence. Ashley (1972) found that the primary source of photosynthate for developing cotton bolls was the boll's subtending leaf and some material was obtained from its carpel walls, associated bracts, and from other leaves on the same sympodium. Muramoto, Hesketh, and Elmore (1967) showed that the rate of CO_2 fixation (assimilate production) by fully enlarged cotton leaves steadily declines from 50 mg CO_2 fixed/dm^2/hr in leaves 1 to 2 days after full expansion to a low rate of 10 mg CO_2 fixed/dm^2/hr at 45 days. The low CO_2-fixing capacity of older leaves might severely limit assimilate availability to maturing bolls from senescing leaves. Morris (1964) suggested that bolls maturing on cotton plants in Uganda received carbohydrate for growth from their associated bracts and capsular wall during the period of rapidly declining CO_2 assimilation in senescing leaves. In this respect, Egamberdyev, Aliev, and Hasyrov (1963) observed maxima at 5 and 45 days after anthesis in the translocation of ^{14}C-assimilate to developing cotton bolls. The purpose of this paper is to report the pattern of incorporation of ^{14}C-assimilate into walls and fibers of developing cotton bolls.

MATERIALS AND METHODS

Plants. The cultivars 'Acala SJ-1,' 'Stoneville 213,' and 'Paymaster III' were grown in the Cotton Variety Test on the Texas A&M University Farm. The individual entries were grown in rows spaced 1 m apart and 10 m long. The cultural practices, including fertilizer, insecticides, herbicides, and irrigations, were conducted to optimize yield of lint. The cultivars yielded 796, 1,318, and 813 kg/ha, respectively. The flowers were tagged on the day of anthesis within the cultivar rows throughout the growing season. Bolls of varying ages (days from anthesis) were sampled from two replicates for boll, seed, and lint measurements. The observations are from individual bolls and the data curves are fit by inspection.

Synthesis of ^{14}C-assimilate and Its Movement to Cotton Bolls. A single plant of Paymaster III with good distribution of developing bolls was selected for the $^{14}CO_2$ feeding experiment. The fruiting positions on this plant are shown in Fig. 1. Note that the bolls of different ages were well distributed throughout the plant. Prior to the treatment a wooden T-frame was constructed over the plant and a 1.2- by 1.8-m polyethylene bag was placed over the plant and sealed at the base of the plant. A small fan was hung from the wooden frame inside the polyethylene bag. To label the assimilates, at 2 PM on September 3, 1971, 5 mCi of $Ba^{14}CO_3$ (sp. act. 197.5 mg/5 mCi) were released inside the bag by adding lactic acid to the radioactive carbonate. The released $^{14}CO_2$ was circulated by the fan. The light intensity at the time of the experiment was 32,280 to 43,040 lux and dropped to less than 5,380 lux at 7 PM. The bag was left over the plant until 9 AM the following morning, at which time the bolls were removed from the plant and brought to the laboratory. The bolls were weighed and separated into boll wall and seeds plus fiber. The boll parts were boiled in 80% ethyl alcohol for 20 min. No attempt was made to extract the insoluble radioactive compounds in the boll wall.

The field experiment was preceded by feeding $^{14}CO_2$ to individual subtending leaves on greenhouse-grown plants and feeding entire greenhouse-grown plants $^{14}CO_2$ in plexiglass chambers and analyzing the radioactivity in the bolls. The six experiments in the greenhouse were trial runs to establish the procedures for the translocation experiment in the field. The data in these experiments paralleled the data in the field experiments but were subjected to the cultural conditions in the greenhouse during the winter months.

Alcohol Extraction. The parts of the cotton boll were extracted five to six times with 80% ethyl alcohol. The material was boiled in alcohol for 10 min during each extraction. To complete the

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[2] Professor of Plant Physiology and Post-Doctoral Fellow, Department of Plant Sciences, Texas A&M University; and Research Geneticist, PSRD, ARS, USDA, College Station, Texas 77843.

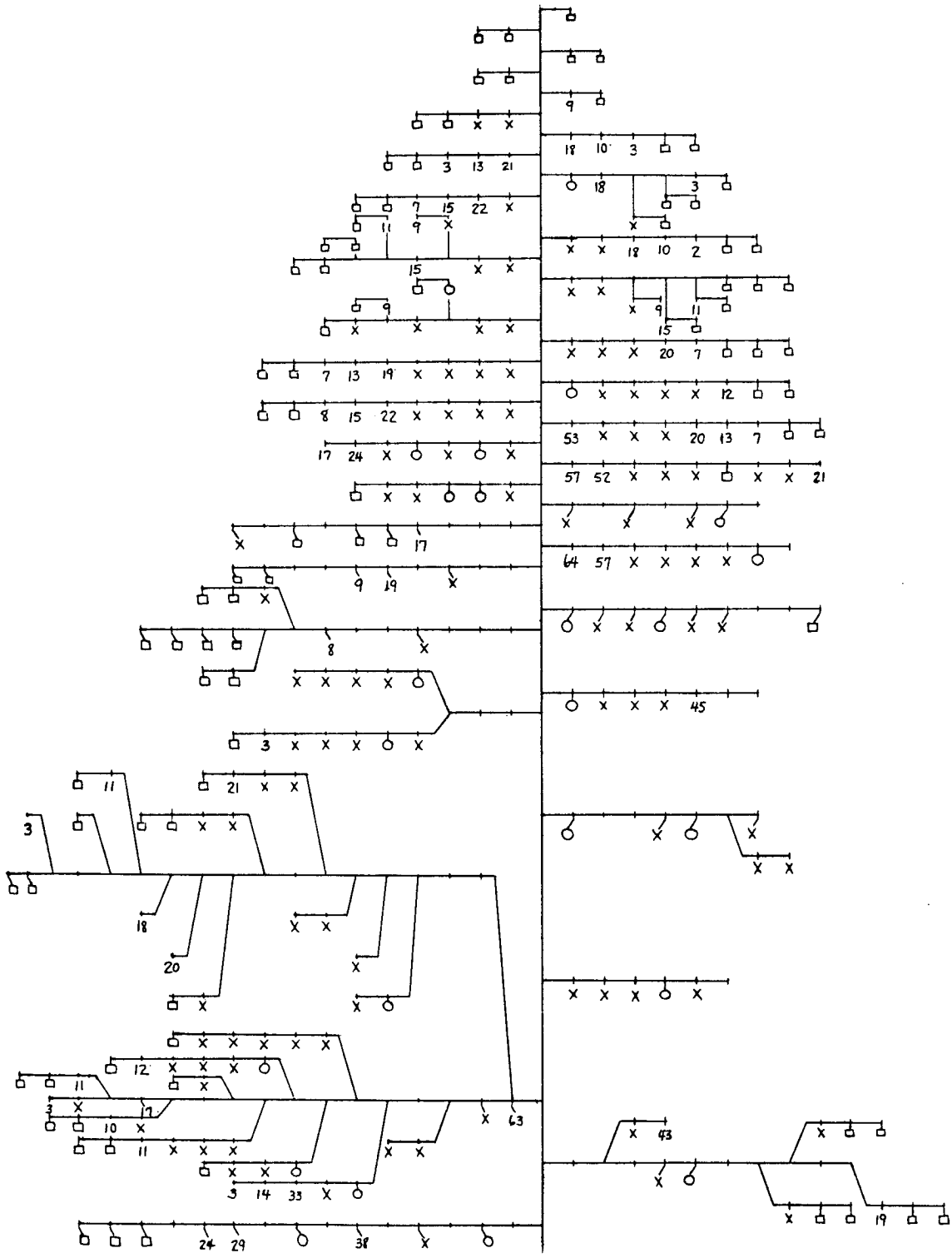

Fig. 1. Distribution of different-age cotton bolls on the experimental plant of Paymaster III on the day of treatment. Numbers indicate the age in days of tagged bolls, circles indicate untagged bolls, x indicates boll positions with no bolls set, squares indicate flower buds, and + indicates nodes on vegetative branches.

extraction the material was boiled three times for 10 min in 95% ethyl alcohol. The alcohol extracts were combined and evaporated to dryness *in vacuo*. The residue was dissolved in H_2O, and the fat-soluble compounds were partitioned into ethyl ether. The H_2O phase was again evaporated to dryness. The *residue* (H_2O soluble compounds) was dissolved in a known volume of H_2O and a 0.1- to 0.5-ml aliquot was assayed for radioactivity.

Analysis of Radioactive Cellulose. The fibers were removed from the seeds and re-extracted with boiling alcohol to remove any traces of compounds adhering to the fibers and to leave only the cellulose incorporated in the fibers. These washes were added to the original extractions of fibers and seeds. The fiber was dried 2 days at 80 C, and the dry weight was determined. [(A known weight of the fiber was placed in a test tube which was placed at 30 C in an H_2O bath for hydrolysis of the cellulose

to glucose. Sulfuric acid (72%) (3 ml/0.3 g dry wt fiber) at 10 to 15 C was added to each sample with a graduated pipet. The sample and acid were mixed with a stirring rod, and initial hydrolysis was allowed to proceed for 1 hr with frequent stirring.)] The hydrolyzate was transferred to a 125- to 500-ml erlenmeyer flask with a known volume of H_2O (84 ml/0.3 g dry wt fiber) and autoclaved for 1 hr at a steam pressure of 15 psi. After the hydrolyzate cooled, a known aliquot was assayed for radioactivity. It had been predetermined that an acid solution did not interfere with the liquid-scintillation assay.

Fiber Length and Dry Weight. Fiber length was determined by the procedure of Gipson and Ray (1969). A locule from a boll was placed in boiling H_2O for 2 to 5 min (2.5% HCl was used for bolls more than 19 days old) to allow the seeds to separate from each other. A seed was placed on the convex side of a watch glass, and the fiber was streamed out with a jet of H_2O. The length of the fiber was measured from the side of the seed. All of the seeds in three locules were measured and an average was reported for the fiber length for a boll. The fiber in all the locules was removed from the seeds and the dry weight of the fiber was determined. Each individual data point in the figures represents the average fiber length and dry weight of a single boll at a given age harvested randomly from different plants in rows of two plot replicates.

Assay of Radioactivity. The amount of radioactivity in the samples was assayed in a Beckman[3] liquid-scintillation-system. Each radioactive sample was added to 15.0 ml of cocktail [(containing 5 g of PPO (diphenyloxazole), 100 g napthalene, 10 ml H_2O, and dioxane to 1 liter)] and dark adapted for several hours prior to counting with ± 0.2% error.

RESULTS

The pattern of incorporation of ^{14}C-photosynthate from a field-grown plant of Paymaster III into the alcohol-soluble compounds of the wall of developing cotton bolls is shown in Fig. 2. Maximum incorporation occurred in bolls that were 8 to 10 days beyond anthesis. Incorporation into boll walls steadily declined after 8 to 10 days to 30 days after anthesis.

Fiber length and fiber dry weights in developing cotton bolls of Paymaster III are summarized in Fig. 3. Fiber elongation was complete 25 to 30 days after anthesis. There was no lag in fiber elongation; whereas, there was a 10- to 12-day lag in the increase in fiber

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

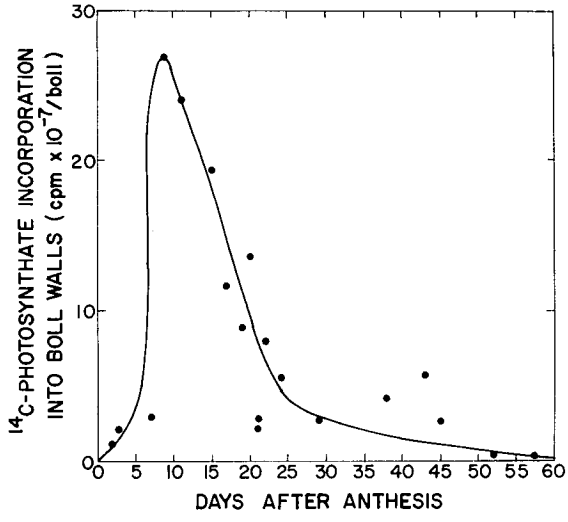

Fig. 2. Incorporation of ^{14}C-photosynthate into the developing cotton boll walls.

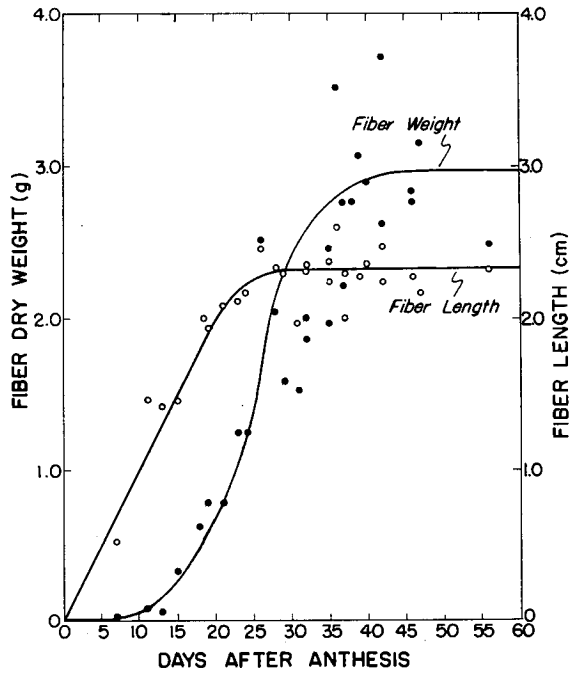

Fig. 3. Fiber length and fiber dry weight in developing cotton bolls of Paymaster III. Each data point represents the average fiber length and dry weight of a single boll at a given age harvested randomly from different plants in rows of two plot replications.

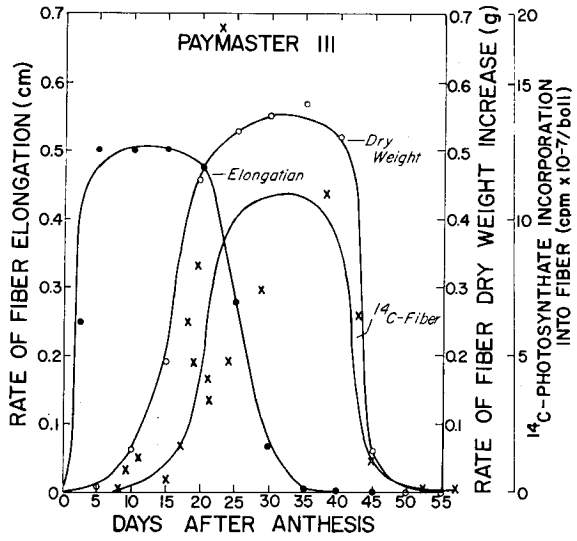

Fig. 4. Rates of fiber elongation and of fiber dry weight increase for each 5-day interval and rate of incorporation of ^{14}C-photosynthate in developing bolls of Paymaster III cotton.

dry weight. Fiber dry weight was maximal 40 to 45 days after anthesis.

Incorporation of ^{14}C-photosynthate into developing fiber showed a lag period of about 10 days (Fig. 4). The most rapid rate of incorporation of ^{14}C-photosynthate into fiber occurred 23 to 40 days after anthesis and coincides with the phase of rapid increase in fiber weight (Fig. 3) and the most rapid rate of dry weight increase (Fig. 4). The decreased incorporation of ^{14}C-assimilates in fiber after 40 days from anthesis coincided with the time of near completion of

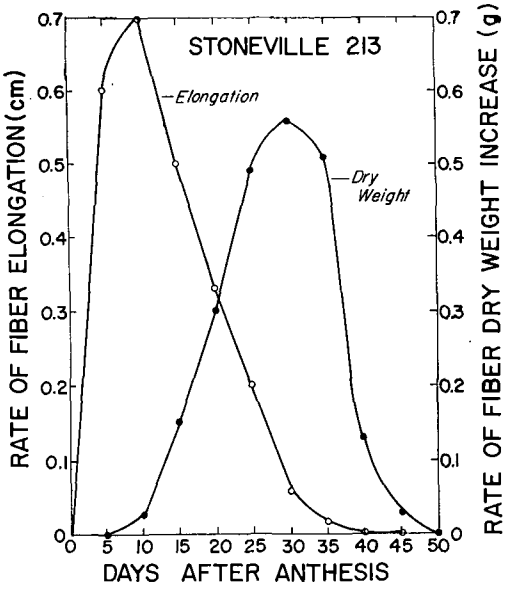

Fig. 5. Rates of fiber elongation and of increase of fiber dry weight for each 5-day interval in developing cotton bolls of Stoneville 213.

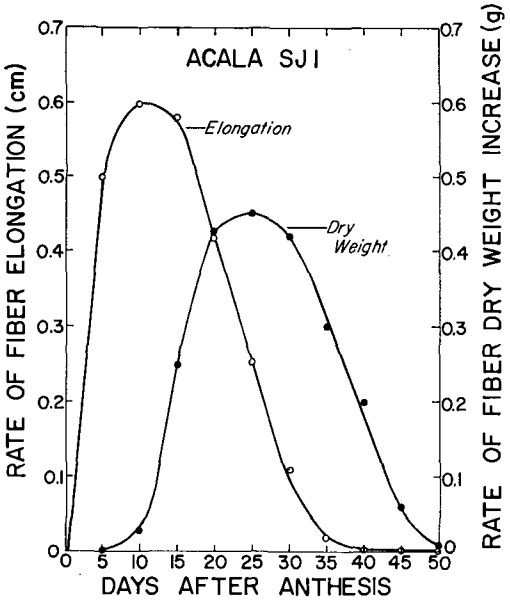

Fig. 6. Rates of fiber elongation and of increase of fiber dry weight for each 5-day interval in developing cotton bolls of Acala SJ-1.

increase of fiber weight. In these experiments and under these cultural conditions, at the end of fiber elongation, i.e., 25 to 30 days after anthesis, fiber weight was between 37 to 59% complete.

The rate of fiber elongation of Stoneville 213 (Fig. 5) and Acala SJ-1 (Fig. 6) peaked at about 10 days after anthesis. The maximum rate of fiber elongation lasted for 5 to 20 days in Paymaster III (Fig. 4). In Stoneville 213 and Acala SJ-1, at the time the rate of fiber elongation was near maximal, the rate of fiber weight increase was just beginning. In Paymaster III, at the time the rate of fiber elongation was near maximal, the rate of fiber weight increase approached its maximum. At the end of the fiber elongation period, the rate of fiber weight increase was at its maximum in all varieties.

DISCUSSION

This paper represents an attempt to obtain quantitative data on the incorporation of ^{14}C-photosynthate into the walls and fiber of developing cotton bolls. No attempt was made to identify the particular source of ^{14}C-photosynthate translocated to the boll sinks. Instead, the entire plant was exposed to $^{14}CO_2$ and the amount of ^{14}C-assimilate that was incorporated into bolls of different ages was recorded. ^{14}C-incorporation into the alcohol soluble compounds of boll walls was maximal at 8 to 10 days after anthesis. Incorporation of ^{14}C-assimilate into walls was complete 30 days after anthesis. This curve was very similar to the curve for rate of fiber elongation in Paymaster III, Acala SJ-1, and Stoneville 213. The curve for ^{14}C-incorporation into fiber in developing bolls of Paymaster III was similar to the bell-shaped curve for the rate of fiber weight increase in developing bolls of Paymaster III, Acala SJ-1, and Stoneville 213. These data show that although incorporation of ^{14}C-photosynthate into fiber in Paymaster III reached a maximum after fiber elongation was complete, there was a substantial incorporation of radioactivity into the fiber and at least 30% of the fiber dry weight was deposited before fiber elongation was complete. These amounts of ^{14}C-photosynthate incorporation and fiber dry weight represent much more cellulose deposition than could be accounted for by primary wall alone. In addition, the increase in fiber dry weight during fiber elongation would be linear if the only cellulose incorporated in the fiber were for elongation, and this was not the observed result. These results do not agree with the earlier conclusions of Anderson and Kerr (1938) who found that some secondary wall thickening in fiber cells occurred before the fiber elongation phase was complete. They concluded, however, that the major phase of secondary wall thickening did not begin until the completion of fiber elongation. These differences could be in part due to the mean temperature differences during the growing season at College Station, Texas, and Raleigh, N.C., or varietal differences.

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