Published January, 1976

Transport of ^{14}C-Assimilates to Cottonseed: Integrity of Funiculus During Seed Filling Stage[1]

C. R. Benedict, R. J. Kohel, and A. M. Schubert[2]

ABSTRACT

Early work suggested the vascular connection from the ovary wall to the ovules in cotton plants (*Gossypium hirsutum* L.) degenerates 32 days after fertilization. A minimum of 50 days after fertilization is required for the maturation of cottonseed. From a yield viewpoint, it is important to establish if leaf assimilates are transported to the developing cotton ovules throughout the seed maturation period.

The incorporation of ^{14}C-assimilates into ovules in different age cotton bolls was measured by: (i) pulse-labeling cotton plants (which contained a wide distribution of different age cotton bolls at a plant stage of maximum fruit load) with $^{14}CO_2$ for 10 min, and (ii) allowing the photosynthetically assimilated $^{14}CO_2$ to be transported to the different age bolls for 20 hours.

The rate of incorporation of ^{14}C-assimilates into fibers in different age cotton bolls was similar to the rate of dry weight increase in the different age bolls. The ^{14}C-assimilate was incorporated into the fibers during a period of active cellulose deposition in these fibers. ^{14}C-assimilate was incorporated into ovule wall, sugars, amino acids, organic acids, oil, and protein in 1- to 45-day-old bolls. This incorporation of photosynthate into the ovules probably reflects transport of ^{14}C-assimilate through the vascular connection. An alternate route of assimilate transport through the carpel wall, into the locule cavity, and absorption by the ovules, is not important because (iii) the carpel wall chloroplasts do not fix $^{14}CO_2$ into ^{14}C-assimilate, and (iv) the transport of leaf and bract ^{14}C-assimilate to the carpel wall is completed in 30- to 35-day-old bolls.

The boll opening period for these cotton plants was 49 to 56 days after anthesis. The results in this paper show that assimilates are transported to cottonseed throughout the maturation period. The majority of this incorporation occurs through the vascular connection and the funiculus does not break 32 days after fertilization.

Additional index words: Gossypium hirsutum L., ^{14}C-assimilate transport, Seed-filling period.

PREVIOUS studies (1, 3, 4, 6, 7, 8, 10) on the anatomy, chemistry, and biochemistry of cottonseed (*Gossypium hirsutum* L.) have provided information on the developmental period for endosperm, embryo and fiber maturation, the linear period of oil and protein deposition, and the changing biochemical events necessary for the regulation of ovule maturation. Yet it has not been established if photosynthetic carbon is deposited in developing ovules throughout the seed maturation period.

Tharp (10) reported that the fertilized ovule reaches its final length and volume 18 to 20 days after fertilization but the maximum weight of the ovule is not obtained until the ovule is detached from the ovary wall, just prior to boll splitting. Anatomical studies (1, 7) have shown the endosperm is fully developed in 20-day-old cotton ovules. Endosperm digestion and embryo enlargement begins 25 to 30 days after anthesis. Benedict et al. (3) and Schubert et al. (8) have shown that upland cotton fibers reach a final length 27 days after anthesis and reach a final dry weight 45 to 50 days after anthesis. Chemical analyses (10) have shown the existence of oil and protein deposition in embryos 25 to 42 days after fertilization. Ihle and Dure (5) and Dure (4) have proposed a temporal map of biochemical and morphological events in developing cotton cotyledons: (i) the funiculus degenerates 32 days after anthesis, (ii) this loss of vascular connection coincides with abscisic acid synthesis in the ovular tissue, and (iii) abscisic acid absorption by the embryo inhibits the translocation of germination mRNA and prevents vivipary.

In south Texas, a minimum of 50 days after anthesis is required for the maturation of upland cottonseed (5). If the funiculus degenerates 32 days after fertilization, the final weight of seedcotton will be determined by the transport of photosynthate to the ovules for only 32 days. This would set the limit on the yield of cotton. The purpose of this research was to establish if leaf assimilates are transported to the developing ovules throughout the seed maturation period.

MATERIALS AND METHODS

Plants. 'Stoneville 213' cotton was grown on the Texas A&M Univ. Farm. Individual entries were grown in rows 50 m long and spaced 1 m apart. Plants were progressively thinned to 1 m between each plant. Cultural practices including applications of fertilizer, herbicides, insecticides, and irrigations were conducted to optimize yield. Flowers were tagged on the day of anthesis throughout the growing season. Bolls of varying ages were sampled from three replicates for fiber dry weight measurements.

Synthesis of ^{14}C-Assimilate and its Transport to Cotton Ovules. Uniform cotton plants, at the stage of maximum boll load, with a wide distribution of developing cotton bolls, were selected for pulse-labeling with $^{14}CO_2$. Plants in adjacent rows were removed to prevent shading of the experimental plants. A polyethylene bag (1.2 × 1.2 × 1.8 m) was placed over each plant and sealed at the base with molding clay and string. 5 mCi of $^{14}CO_2$ was released inside the bag by injecting 5 ml of lactic acid through a hyperdermic syringe onto $NaH^{14}CO_3$ (sp. act. 5 mCi / 89.0 mg $NaH^{14}CO_3$) in a 50 ml beaker inside the bag. The $^{14}CO_2$ was circulated inside each bag with a fan operated by a 6 V battery inside the bag. The experiments were carried out between 1 p.m. and 2 p.m. on a cloudless day. Light intensity was 0.23 × 10^4 microeinsteins m^{-2} sec^{-1} as measured with a LI-185 Lambda Quantum[3] instrument. Each plant was exposed

[1] This study was supported by the USDA with funds made available through Cotton Incorporated. Received 4 June 1975.

[2] Professor of plant physiology, Dep. of Plant Sciences, Texas A&M Univ., College Station, TX 77843; research geneticist, ARS, USDA, College Station, TX 77843; and former graduate assistant (now research fellow), Dep. of Agronomy, Univ. of Georgia, Athens, GA 30602.

[3] Mention of a trademark name or a proprietary product does not constitute endorsement by USDA or Texas A&M Univ. and does not imply its approval to the exclusion of other products that also may be suitable.

to $^{14}CO_2$ for 10 min, and the bag removed. Translocation of the ^{14}C-assimilate from the photosynthetic sources to the developing cotton bolls was allowed for 20 hours. At the end of the translocation period, the bolls were removed from the plants. The individual bolls were slit open and the ovules with attached fibers placed in beakers of bolling 80% ethyl alcohol. The ovules were boiled for 15 min and then brought to the laboratory for analysis. Determinations were made of the amount of ^{14}C-assimilates incorporated into oil, neutral sugars, amino acids, organic acids, protein, and fiber from ovules in different age cotton bolls. Determinations were made on 14 different age bolls from two plants.

Metabolism of ^{14}C-Assimilates in the Ovules. We have previously shown that 90 to 95% of ^{14}C-assimilates are exported from mature cotton leaves in a 20 hour translocation period (2) but there is no information on the metabolism of ^{14}C-assimilates after they are deposited in the cotton bolls. A single cotton plant with a number of 29- to 35-day-old bolls was selected for pulse-labeling with $^{14}CO_2$. The plant was exposed to 5 mCi of circulating $^{14}CO_2$ for 10 min as described in the previous section. Translocation of ^{14}C-assimilates from photosynthetic sources to boll sinks was allowed for 20 hours.

The metabolism of the ^{14}C-assimilates in the cotton ovules was monitored for 6 days. Every 24 hours, two replicate 35-day-old cotton bolls were removed from the plant and the amount of radioactivity in the H_2O soluble compounds, fiber, and oil in the ovules was determined.

Ovule Extraction. The ovules (including fibers) were extracted four times in boiling 80% ethyl alcohol and four times in boiling 95% ethyl alcohol. Fibers were removed from the ovules and re-extracted with 80 and 95% ethyl alcohol to remove all traces of compounds adhering to the fiber. These fiber extractions were added to the original ovular extractions.

Oil Analysis. Ovular alcohol extractions were evaporated to dryness in vacuo. The water- and fat-soluble compounds in this residue were partitioned into water and ethyl ether. The ether fraction was removed and brought to a known volume. An aliquot was assayed for radioactivity in the oil.

Sugar, Amino Acid, and Organic Acid Analysis. The above H_2O soluble fraction was evaporated to dryness in the presence of a small amount of formic acid (to remove any traces of $^{14}CO_2$ dissolved in the H_2O). The dry residue was dissolved in H_2O and quantitatively passed through columns of Dowex-50 (H+) resin and Dowex-1 (formate) resin. Effluent from Dowex-1 resin contained the neutral sugars. Amino acids were displaced from Dowex-50 resins with 1 N NH_4OH. Organic acids were displaced from the Dowex-1 resins with 8 N formic acid. Each fraction was evaporated to dryness, dissolved in a known volume of H_2O, and assayed for radioactivity.

Cellulose Analysis. Fiber that had been removed from the ovules was dried 24 hours at 80 C and the dry weight was determined. A known weight of fiber was placed in a test tube. Three ml of 72% sulfuric acid per 0.3 g dry wt of fiber was added to each sample with a graduated pipet at 10 to 15 C. The sample and acid were mixed with a stirring rod. Initial hydrolysis was for 1 hour at 30 C with frequent stirring. The hydrolysates were transferred to a 125 to 500 ml erlenmeyer flask with a known volume of H_2O (84 ml / 0.3 g dry wt of fiber) and autoclaved 1 hour at a steam pressure of 15 psi. After the hydrolysate cooled, an aliquot was assayed for radioactivity. It had been predetermined that an acid solution did not interfere with the liquid-scintillation assay.

Insoluble Residue Analysis. Following the removal of the fibers and exhaustive alcohol extraction of the ovules, the insoluble residue was heated to dryness at 80 C for 24 hours. A 25 mg sample of dry residue was hydrolyzed to H_2O soluble compounds by the acid digestion procedure in the previous section. Composition of the ovular insoluble residue was protein and seed hull (8), but the relative percentages of these constituents changed with seed maturation.

Fiber Dry Weight. Fiber in all of the locules was removed from the seed, and the dry weight of the fiber determined. Each individual data point in the figure represents the average fiber dry weight of a single boll at a given age harvested randomly from different plants. Fiber dry weight was determined for three replicate bolls.

Assay of Radioactivity. The amount of radioactivity in the samples was assayed in a beckman liquid-scintillation-system. Each radioactive sample was added to 15.0 ml of cocktail [(containing 5 g PPO (diphenyloxazole), 100 g of napthalene, 10 ml H_2O and dioxane to 1 liter)] and dark adapted for several hours prior to counting with ± 2% error.

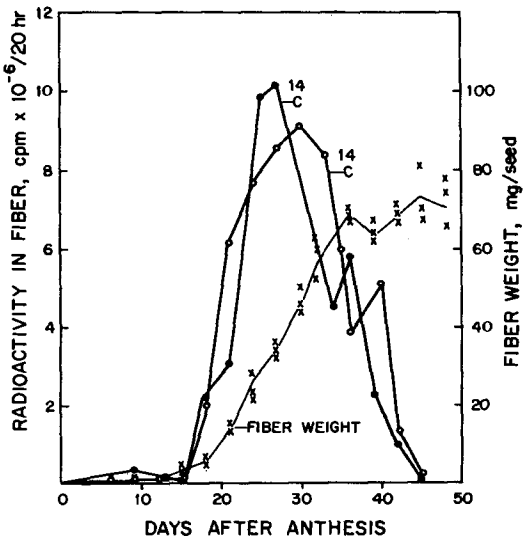

Fig. 1. Development of fiber dry wt compared to rate of incorporation of ^{14}C-assimilates into fibers in different age ovules. Two ^{14}C-curves are data from two replicate plants.

RESULTS AND DISCUSSION

Abscisic acid synthesis may accompany the loss of the vascular connection in cotton ovules 32 days after anthesis (4, 5). The synthesis of this hormone by the ovular tissue may prevent vivipary and allow the completion of seed maturation. These postulates have been formulated into a working model to explain cotton embryogenesis but they raise serious questions relative to the yield of seedcotton. If the funiculus breaks 32 days after fertilization, the yield of seedcotton will be determined by the transport of photosynthate to the ovules for only 32 days. This will limit the yield of cotton unless some other route exists to supply photosynthetic carbon to the ovules for the remainder of the maturation period.

The results in Fig. 1 show the rate of ^{14}C-assimilate that is incorporated into cotton fibers in different age cotton bolls in two plants following a pulse-labeling of the photosynthate with $^{14}CO_2$. Significant amounts of radioactivity are incorporated into fibers in 19- to 45-day-old bolls. The greatest rate of ^{14}C incorporation was into fibers in 26- to 30-day-old bolls. Data in Fig. 1 also show that the majority of ^{14}C-photosynthate is incorporated into the cotton fibers during the linear period of fiber dry weight accumulation. It should be noted that ^{14}C-assimilate is incorporated into the primary wall of fiber prior to 19 days but this amount of incorporation is small compared to the amount which is incorporated into the fiber during secondary wall formation.

The data in Fig. 2 show plots of the amount of ^{14}C-assimilate incorporated into fibers in different age bolls in a 20 hour translocation period (i.e. cpm/20 hour) and a curve of fiber dry weight increases in different age cotton bolls. The curve for fiber dry weight increase results from plotting fiber dry weight increases between cotton bolls 6, 9, 12, 15,

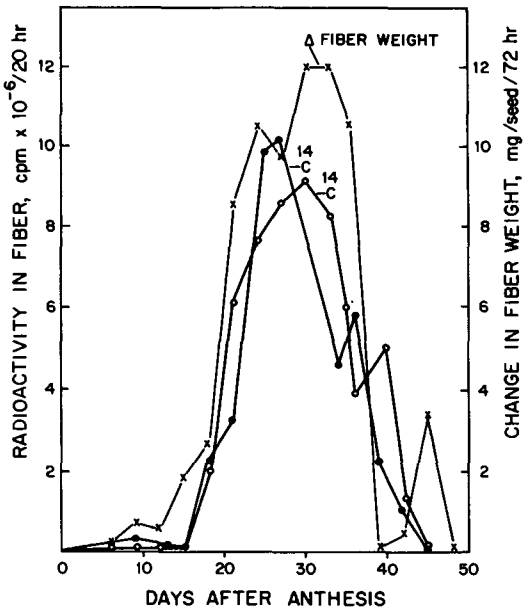

Fig. 2. Rate of development of fiber dry wt compared to rate of incorporation of ^{14}C-assimilates into fibers in different age ovules.

18, 21, 24, 27, 30, 33, 36, 39, 42, 45, and 48 days old. This curve is very similar to the rate curve for lint weight increases calculated in a preceding paper (8). Previously the dry weight of lint fiber/seed vs. boll age was fitted to a growth curve $W = A / (1 + BP^x)$. The rate curve for lint weight increase plotted against boll age was determined by calculating the derivative of the growth curve of dry weight of lint fiber/seed vs. boll age as defined by this equation $dW/dx = -[ABP^x (\ln P)/(1 + BP^x)^2]$.

where

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

$$W_x = o = A / (1 + B)$$

or

$$B = (A / W_x = o) - 1$$

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

The rate curve for ^{14}C-assimilate incorporation into fiber in different age bolls (Fig. 2) is similar to the rate curve for lint weight increases vs. boll age. The data show that the rate of ^{14}C-assimilate incorporation into fibers in different age bolls is low when the change in fiber dry weight, i.e. deposition of photosynthate into fiber cellulose is low and high when the rate of deposition of photosynthate into fiber cellulose is high. These results show that the determination of ^{14}C-assimilate incorporation into fibers of different age cotton bolls reflects the actual process of cellulose synthesis in these fibers.

The results in Fig. 3 show the rate of ^{14}C-assimilate incorporation into ovule oil in different age cotton bolls. There is a minor rate of ^{14}C-assimilate incorporated into the oil in 5- to 18-day-old bolls. The

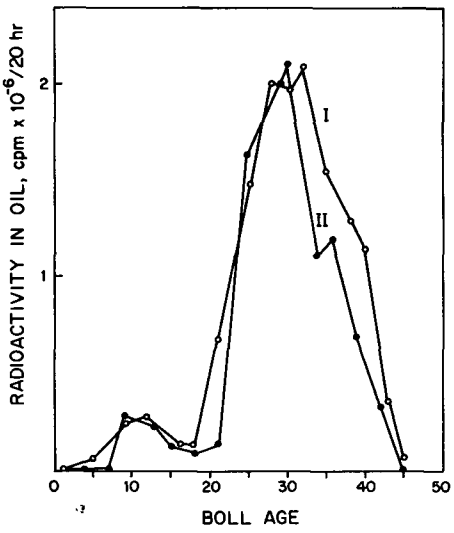

Fig. 3. Rate of incorporation of ^{14}C-assimilates into oil in different age ovules.

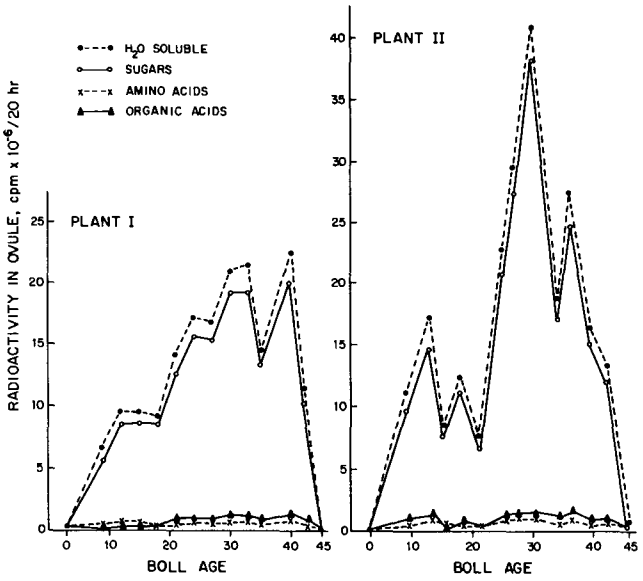

Fig. 4. Rate of incorporation of ^{14}C-assimilates into H_2O soluble compounds, sugars, amino acids, and organic acids in different age cotton ovules.

rate of ^{14}C-assimilate incorporation into the ovule oil is greatest in bolls 19 to 45 days old.

The data in Fig. 4 show the rate of ^{14}C-assimilate incorporated into H_2O soluble compounds, sugars, amino acids, and organic acids in developing ovules. The ^{14}C-assimilate is incorporated into these ovule compounds in 1- to 45-day-old bolls. The predominant H_2O soluble compound has been identified by two-dimensional chromatography as ^{14}C-glucose.

The data in Fig. 5 show the rate of incorporation of ^{14}C-assimilate into the insoluble ovule compounds in different age cotton bolls. The insoluble compounds are primarily protein and seed coat (9). The ^{14}C-assimilate is incorporated into these components in 1- to 45-day-old bolls.

The data in Fig. 6 is a composite drawing of the amount of ^{14}C-assimilate incorporated into sugars,

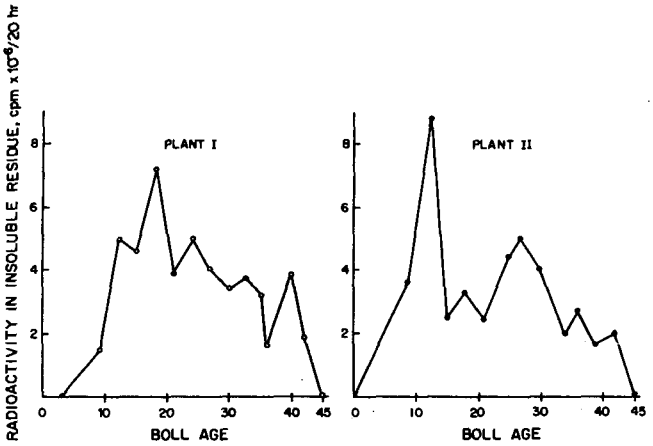

Fig. 5. Rate of incorporation of ^{14}C-assimilates into insoluble residue in different age cotton ovules.

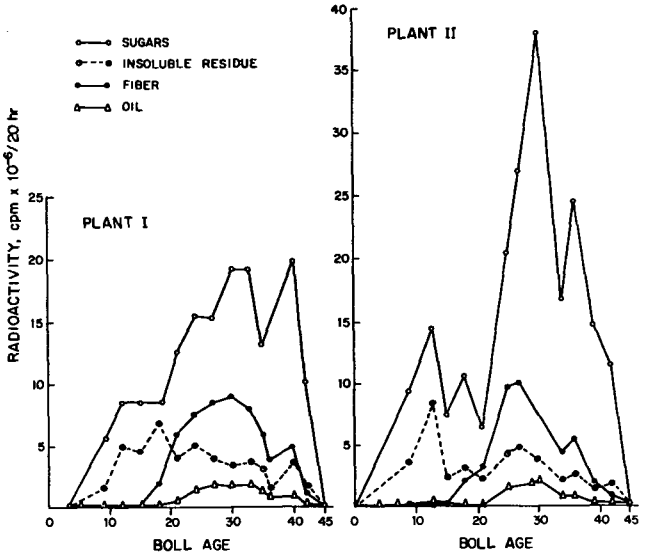

Fig. 6. Rate of incorporation of ^{14}C-assimilates into sugars, insoluble residue, fibers, and oil in different age cotton ovules.

fiber, oil, and insoluble residue in developing ovules. In a 30-day-old cotton boll in a 20 hour translocation period, there is between 57 and 67% of the total radioactivity in the ovules in glucose.

The data in Figs. 1 to 6 show that ^{14}C-assimilate is incorporated into ovules throughout the seed maturation period. These experiments have not, however, directly measured the amount of ^{14}C-assimilate incorporated into the ovules through the funiculus. It is conceivable that assimilate could be dumped into a reservoir in the locular cavity through the carpel wall or ovule wall. The carbon from this reservoir could be absorbed by the ovules. In separate experiments we have noted that injection of ^{14}C-glucose into the locular cavity of cotton bolls leads to isotope absorption by the ovules. Glucose is absorbed and incorporated into cellulose by excised locules incubated with ^{14}C-glucose. Therefore some attention should be given to the existence of an alternate route for assimilate incorporation into ovules.

In previous work (2, 3) we have shown that (i) $^{14}CO_2$ fixation by the chloroplasts of the carpel wall

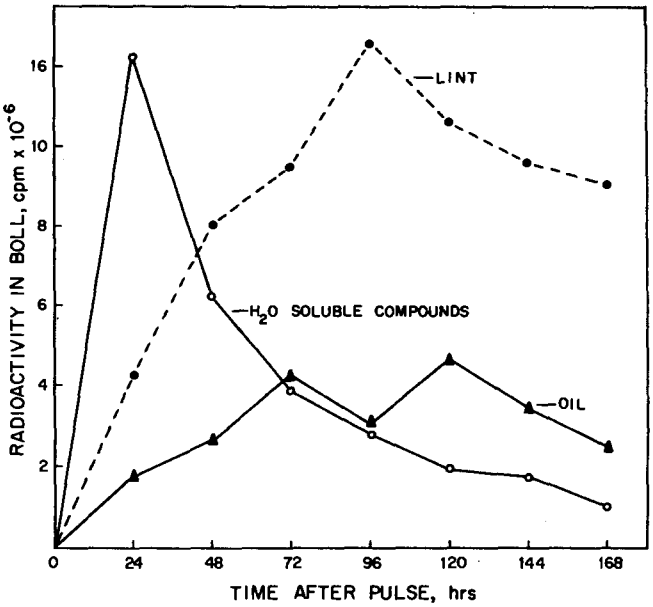

Fig. 7. Metabolism of ^{14}C-assimilates in developing cotton ovules. Data points are the mean of radioactivity in two replicate cotton bolls.

into ^{14}C-assimilate is low or negligible and (ii) the transport of leaf or bract ^{14}C-assimilate to the carpel wall is complete in 30- to 35-day-old bolls. These experiments seem to discount the route of incorporation of ^{14}C-assimilate into ovules, throughout the seed maturation period, through the carpel wall and locular cavity.

The last possible route for the incorporation of assimilate into the locules, after a possible break in the funiculus, is through a reservoir in the locular cavity, which was made prior to the severing of the vascular connection. This route is made more plausible when one examines the growth curves for fibers, seed, and cotyledons (4, 6, 8, 10) for the period after 32 days. If the funiculas breaks at 32 days, approximately one-half of the dry weight of the lint and seed must come from a source outside the detached ovule. The only source would be a carbon reservoir in the locular cavity deposited there prior to 32 days.

The data in Fig. 4 show the majority of the water-soluble compounds isolated from locules (containing ovules and lint) is glucose. No effort was made to determine whether the glucose is inside or outside the ovule. The metabolism of this ^{14}C-glucose in 30-day-old cotton bolls is shown in Fig. 7. Twenty-four hours after pulse-labeling a cotton plant with $^{14}CO_2$, 71% of the label in the ovules is in ^{14}C-glucose. This ^{14}C-glucose disappears after 3 to 4 days with a concomittant increase in ^{14}C-oil and ^{14}C-lint. This reservoir of glucose could not support the increase in the dry weight of the lint or seed following the funiculus degeneration at 32 days after anthesis.

Therefore the results in this paper show ^{14}C-assimilate is incorporated into ovules throughout the seed maturation period. The majority of ^{14}C-assimilate is incorporated into the ovule through the funiculus and the vascular connection does not break in cotton bolls 32 days after anthesis.

REFERENCES

1. Baranov, T. A., and A. M. Maltzer. 1937. The structural and development of the cotton plants. Laboratory Cytology and Anatomy of the Central Plant Breeding Station, Moscow-Leningrad (English Translation).
2. Benedict, C. R., and R. J. Kohel. 1975. Export of ^{14}C-assimilates in cotton leaves. Crop Sci. 15:367-372.
3. ———, R. H. Smith, and R. J. Kohel. Incorporation of ^{14}C-photosynthate into developing cotton bolls, *Gossypium hirsutum* L. Crop Sci. 13:88-91.
4. Dure, L. S. 1975. Seed formation. Annu. Rev. Plant Physiol. 26:259-278.
5. Gerard, C. J., and S. A. Reeves. 1973. Variety, row spacing and date of planting influences on economical cotton production on clay soils in lower Rio Grande Valley. Texas Agric. Exp. Stn., Tech. Rep. 73-1.
6. Ihle, J. N., and L. Dure III. 1972. The developmental biochemistry of cottonseed embryogenesis and germination. J. Biol. Chem. 247:5048-5055.
7. Leahy, J. 1948. Structure of the cottonseed. P. 105-116. *In* A. E. Bailey (ed.) Cottonseed and cottonseed products. Interscience Publishers, Inc., New York.
8. Schubert, A. M., C. R. Benedict, J. D. Berlin, and R. J. Kohel. 1973. Cotton fiber development-kinetics of cell elongation and secondary wall thickening. Crop Sci. 13:704-709.
9. Tharp, W. H. 1948. Cottonseed composition-relation to variety, maturity, and environment of the plant. P. 117-156. *In* A. E. Bailey (ed.) Cotton and cottonseed products. Interscience Publishers, Inc., New York.
10. ———. 1965. The cotton plant. How it grows and why its growth varies. Agricultural Handbook No. 178, ARS-USDA.