Published May, 1975

Export of ^{14}C-Assimilates in Cotton Leaves[1]

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ABSTRACT

A study was made of the export of ^{14}C-assimilate in mature leaves of 'Stoneville 213' cotton (*Gossypium hirsutum* L.). Fruiting branches and subtending leaves of field grown plants were pulse-labeled with $^{14}CO_2$ for 5 min at about mid-day. The amount of ^{14}C-assimilate transported to the cotton boll from the subtending leaves, bracts or boll wall, as well as the amount of ^{14}C-assimilate remaining in the leaves and bracts, were determined over a 22-hour period. During this translocation period, leaves and bracts export ^{14}C-assimilate to the boll wall, ovules and lint; whereas the boll wall does not export ^{14}C-assimilate. The leaves are the dominant source of assimilate for cotton bolls throughout the season.

There is a differential rate of export and retention of H_2O soluble and insoluble (starch) assimilate from the leaves and bracts. There is an initial rapid phase of transport of H_2O soluble compounds from both leaf and bract sources. About 80% of these compounds disappear 6 hours after exposing the sources to $^{14}CO_2$. After 22 hours, over 90% of this pool is depleted. In contrast, there is a retention of ^{14}C in the starch fraction up to 6 hours after pulse-labeling the sources with $^{14}CO_2$. After 6 hours, there is a slow export of ^{14}C-assimilate from the starch fraction stored in the sources. Cotton leaves export assimilate stored as starch slowly. At the end of 22 hours, 90 to 95% of the ^{14}C-assimilate disappears from mature leaves and bracts.

Additional index words: Translocation, ^{14}C-photosynthate, Source-sink.

PREVIOUS studies have described the translocation of leaf assimilate in cotton (*Gossypium hirsutum* L.) plants. Brown (4) found that maturing cotton bolls receive assimilate from the subtending leaves, associated bracts, boll wall, leaf subtending the sympodium, and leaves higher up on the same side of the stem. Ashley (1) found the primary source of photosynthate for the developing cotton boll was the subtending leaf. Muramoto, Hesketh, and Elmore (11) have shown that the rate of CO_2 fixation by fully enlarged cotton leaves, steadily declines from 50 mg CO_2 fixed/dm^2·hr in leaves 1 to 2 days after expansion to a low rate of 10 mg CO_2 fixed/dm^2·hr at 45 days after expansion. Morris (10) has suggested that bolls received carbohydrate for growth from their associated bracts and capsular wall during the period of rapidly declining CO_2 assimilation in the subtending leaves.

Ashley (1) has noted that 43% of the photosynthate remained in the leaf following 24 hours of translocation. Brown (5) reported that the proportion of labeled assimilates, which are exported from cotton leaves, reached a maximum of 65% in leaves 21 to 42 days after unrolling. These studies showed a retention of 35 to 43% of the daily photosynthate in mature cotton leaves. Other plants retain different amounts of assimilate. Corn (*Zea mays* L.) translocates assimilate rapidly and completely out of the leaves (7). Sugarcane (*Saccharum officinarum* L.) translocates 80% of the ^{14}C-assimilate, labeled by an initial pulse of $^{14}CO_2$, in 4 hours (6). Other tropical grasses export 70% of assimilate within 6 hours of assimilation; whereas tomato (*Lycopersicon esculentum*), castor bean (*Ricinus communis* L.), tobacco (*Nicotiana tobacum* L.), and soybean (*Glycine max* L.), translocates only 45 to 50% of the assimilate in 6 hours (8). In those species with a low rate of assimilate export, there was a high retention of ^{14}C-assimilate as starch for the 6 hours of the experiment. There are no studies relating the export of H_2O soluble compounds and starch in dicotyledonous crop plants.

The purpose of this work was to establish the primary source of photosynthate for cotton bolls throughout the fruit maturation period and to study the export of H_2O soluble assimilate and H_2O insoluble assimilate (starch) in mature cotton leaves.

MATERIALS AND METHODS

Plants. 'Stoneville 213' cotton was grown in the cotton variety test on the Tex. A&M U. farm. The individual entries were grown in rows spaced 1 m apart and 50 m-long. The plants were thinned to 1 m between each plant. The cultural practices including fertilizer, herbicides, insecticides, and irrigations, were conducted to optimize yield. The flowers were tagged on the day of anthesis throughout the growing season.

Photosynthate Source for Cotton Bolls. Four plants were selected for uniformity (i.e., containing different age bolls on a single fruiting branch midway on each plant). Each sympodium contained at least four bolls which were between 20 to 40-days old.

The entire fruiting branch was enclosed in a polyethylene bag and sealed, around its base, with molding clay and string. The other adjacent branches were tied back to prevent shading of individual leaves, bracts, or boll walls. $^{14}CO_2$ (1mCi) was released inside the bag by injecting 2.0 ml of lactic acid onto $NAH^{14}CO_3$ (sp act 1 mCi/18.6 mg $NaH^{14}CO_3$). The $^{14}CO_2$ was circulated inside the bag with a fan. Each leaf on the sympodium was oriented toward the sun. Each experiment was carried out between 11 AM and 2 PM on a cloudless day. The light intensity was 0.3 to 0.5 $\times$ 10^4 μ Einsteins m^{-2} sec^{-1} as measured with a LI-185 Lambda Quantum instrument. The leaves were exposed to a 5 min pulse of $^{14}CO_2$ and the polyethylene bag removed. The sources were allowed to translocate the ^{14}C- assimilate for 22 hours.

This experiment included measuring the amount of ^{14}C-assimilate translocated to different age cotton bolls on fruiting branches containing: i) intact branches with leaves, bracts, and bolls; ii) branches containing only leaves and bolls; iii) branches containing only bracts and bolls; and iv) branches containing only bolls.

At the end of the translocation period, the bolls were removed from the plant. The boll was slit-open, and the boll wall and the ovules containing the lint fibers were placed in separate beakers of boiling 80% ethyl alcohol. A determination was made of the amount of radioactivity translocated from the leaves, bracts, or boll wall to the lint, alcohol, or H_2O soluble compounds in the ovules and alcohol or H_2O soluble compounds in the boll wall.

Characterization of Photosynthate in Sources. A fruiting branch containing at least five cotton bolls between 10 to 37-

[1] This study was supported by the USDA, with funds made available through Cotton Incorporated. Received Oct. 19,1974.

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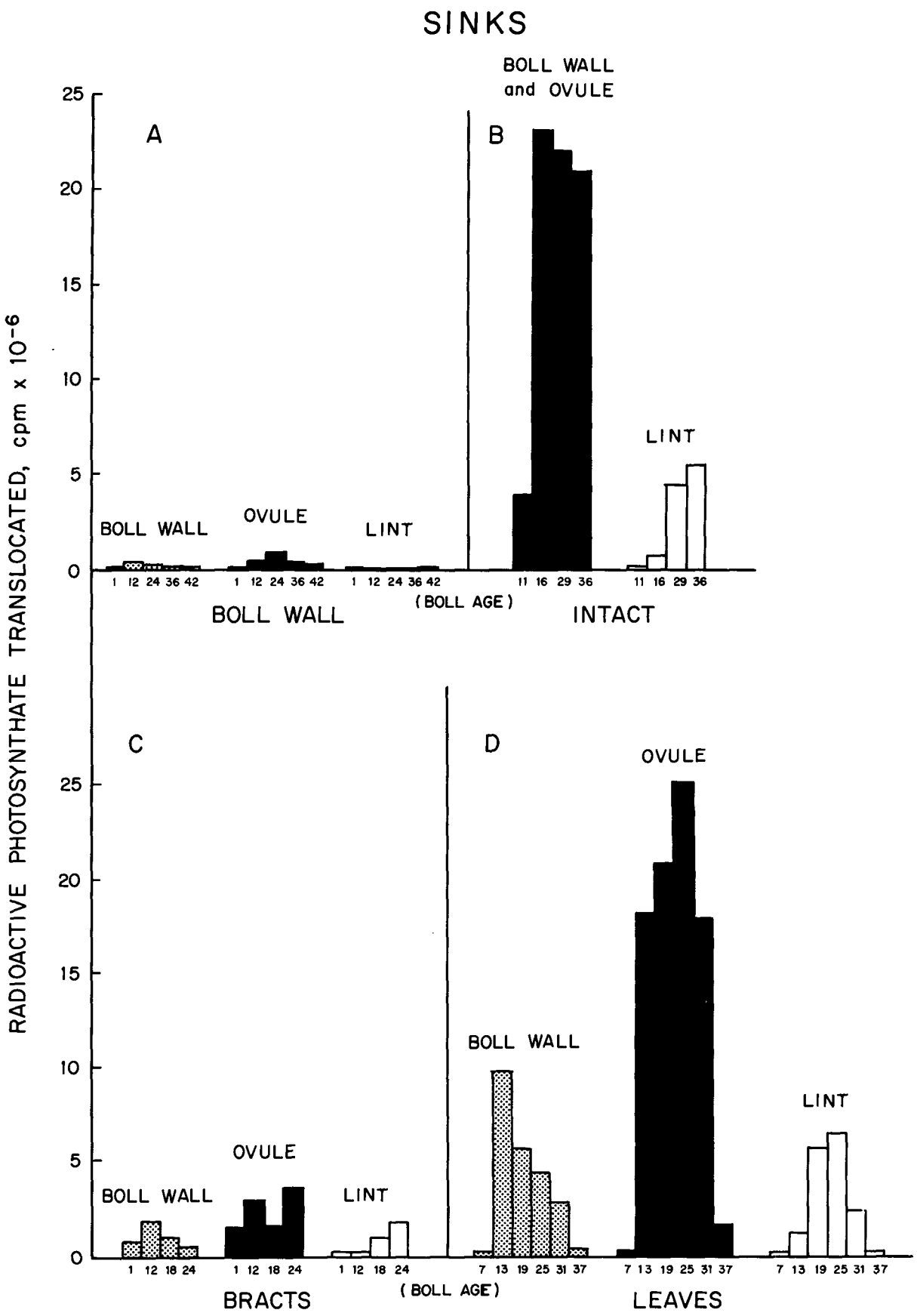

Fig. 1. Translocation of ^{14}C-assimilate from sources to different age cotton boll sinks. The amount of ^{14}C assimilate transported from: A) cotton boll wall to the ovules and lint; B) all photosynthetic sources on an intact fruiting branch to the cotton boll wall, ovules, and lint sinks; C) bracts to the boll wall, ovules, and lint sinks; and D) subtending leaves to boll wall, ovules, and lint sinks.

days old was enclosed in a polyethylene bag as described above. The leaves and bracts were exposed to a 5 min pulse of 1 mCi of $^{14}CO_2$. At the end of the fixation period, the leaves and bracts were placed in separate beakers of boiling 80% ethyl alcohol. The amount of radioactive CO_2 incorporated into alcohol soluble (referred to as H_2O soluble) and alcohol insoluble (starch) assimilates was determined.

Retention of ^{14}C-Assimilates in the Subtending Leaves and Bracts. Five plants were selected for uniformity (i.e., containing different age bolls on a single fruiting branch midway on each plant) as previously described. The leaves and bracts were exposed to a 5 min pulse labeling with 1 mCi of $^{14}CO_2$. The leaves and bracts on each branch were allowed to translocate the ^{14}C-assimilate for 0 and 15 min and 1, 6, and 22 hours. At the end of each translocation period, the bolls and subtending leaves and bracts were separated from the branch and placed in separate beakers of boiling 80% ethyl alcohol. The amount of radioactivity in leaf and bract soluble and insoluble ^{14}C-assimilate, lint and soluble compounds, boll wall, and ovules, was determined. Each data point in this experiment is the mean of four entries of leaves, bracts, or bolls along a single fruiting branch.

Retention of ^{14}C-Assimilates in the Subtending Leaves. In August, eighteen plants were selected for uniformity from 50-m rows. Each plant contained at least one mature leaf, midway on a fruiting branch, subtending a 30-day-old cotton boll. Each subtending leaf was enclosed in a polyethylene bag which was sealed around the petiole with molding clay and string. 40 μCi of $^{14}CO_2$ was released from $NaH^{14}CO_3$ (sp act 1 mCi/18.6 mg) with lactic acid inside the bag. The released $^{14}CO_2$ was circulated with a fan. Each leaf was pulsed with $^{14}CO_2$ for 5 min. This experiment was done from 1:30 to 3:30 PM. The light intensity was 0.3 to 0.4 $\times$ 10^4 μ Einsteins m^{-2} sec^{-1}. After the 5 min pulse, the polyethylene bag was removed. The subtending leaves were allowed to export the ^{14}C-assimilate for 0 and 15 min and 1, 3, 6, and 22 hours following the pulse labeling period.

Following each translocation period the leaves were placed in boiling 80% ethyl alcohol. The amount of ^{14}C retained in the alcohol soluble and alcohol insoluble was determined. Each data point is the mean of two replicates. The radioactive sugars, organic acids, and amino acids within the H_2O soluble fraction were separated by resin chromatography as previously described (2).

In south Texas, cotton bolls of short staple cotton require 55 days following anthesis for maturity. Leaves subtending a 55-day-old boll would be 75 days. Leaves subtending 30-day-old bolls would be 50-days-old. The linear phase of cellulose deposition in short staple cotton fibers is from 17 to 45 days after anthesis (3). Leaves subtending bolls, which are in an active stage of cellulose synthesis, are not 'old' cotton leaves. The export of assimilate in these leaves is typical of mature cotton leaves.

Assay of the Amount of Radioactivity in Alcohol Soluble and Alcohol Insoluble Assimilate. The leaves, bracts, boll walls, and ovules, were extracted six times with boiling 80% ethyl alcohol and three times with boiling 95% ethyl alcohol. Each time the material was boiled for 10 to 15 min. This procedure extracted the alcohol soluble compounds. The individual extractions were pooled and evaporated to dryness (*in vacuo*). The residue was dissolved in 10 ml of H_2O, and a 0.1 to 0.2 ml aliquot was assayed for radioactivity.

After the alcohol soluble compounds had been extracted from the leaves and bracts, the alcohol insoluble starch was extracted by the following procedure. The leaves were thoroughly dried at 80 to 90 C and ground to a fine powder in a mortor. The ground leaf sample (25 mg) was placed with 2.0 ml of H_2O in a test tube in a boiling H_2O bath for 60 min. The tube contents were cooled to room temperature. 10 mg of α-amylase was added to each tube, and the contents gently stirred for 3 hours at 50 C. Following the enzymatic digestion period, the contents were heated at 100 C to denature the protein, centrifuged, and the soluble supernatant fraction removed. The pellet was washed three times with H_2O, and the original supernatant and washings combined. The volume was measured and a 0.2 ml aliquot assayed for radioactivity. The only radioactive compound in this digestion fraction was identified, by chromotography and autoradiography, as ^{14}C-glucose. The radioactivity in this fraction is equated to leaf ^{14}C-starch. The remaining leaf residue was acid hydrolyzed by the procedure for acid hydrolysis of cellulose (see below). The ^{14}C recovered in the acid hydrolyzate represented ^{14}C-insoluble leaf residue.

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 fibers were 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 hour 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 hour 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.

Assay of Radioactivity. The amount of radioactivity in the samples were assayed in a Beckman liquid scintillation spectrometer. Each radioactive sample was added to 15.0 ml of solution [containing 5 g PPO (diphenyl oxazole), 100 g napthalene, 10 ml H_2O, and dioxane to 1 liter] and dark adapted for several hours prior to counting with a $\pm$ 0.2% error.

RESULTS

Photosynthate Source for Cotton Bolls. Morris (10) has suggested a shift in the source of photosynthate for developing cotton bolls from subtending leaf to

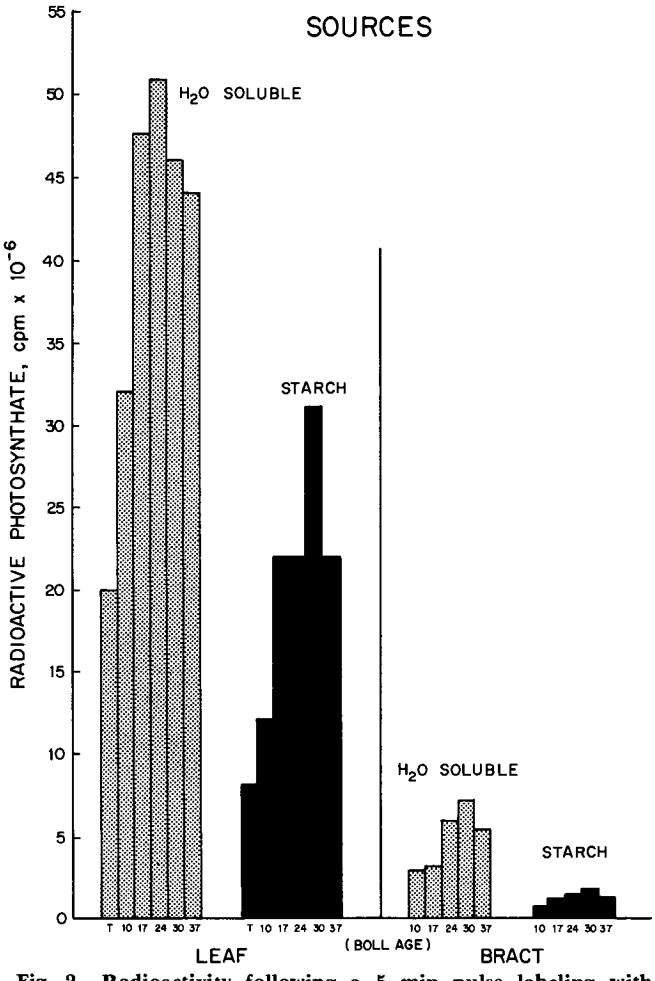

Fig. 2. Radioactivity following a 5 min pulse labeling with $^{14}CO_2$ (T = terminal leaf on the fruiting branch).

boll wall and bract, which occurs during the decline in CO_2 fixation in subtending leaves. We have determined the relative contribution of subtending leaves, bracts, and boll wall in furnishing assimilate to different parts of the cotton boll throughout boll maturation. The contribution of the different sources was determined by labeling the assimilate on a fruiting branch which contained: i) all of the photosynthetic sources, ii) only the subtending leaves with boll, iii) only the bracts with boll, and iv) only the cotton boll. The amount of ^{14}C-assimilate transported to the cotton boll, seed, and lint was then determined. As shown in Fig. 1, compared to the amount of ^{14}C-assimilate transported to the boll sinks from all sources (i.e., intact branch), the major photosynthetic source for cotton bolls of all ages is the subtending leaves. The associated bracts furnish only 5 to 10% of the photosynthate for cotton bolls 1 to 24 days after anthesis. In cotton bolls of all ages, the boll wall contributes little photosynthate for their growth.

Characterization of Photosynthate in Sources. The amount of radioactivity in the H_2O soluble and starch fractions in subtending leaves and bracts following a 5 min pulse labeling with $^{14}CO_2$ is shown in Fig. 2. In leaves subtending different age cotton bolls, there is 20 to 51 $\times$ 10^6 cpm in H_2O soluble compounds and 8 to 31 $\times$ 10^6 cpm in starch. Separate calculations show that the amount of ^{14}C-H_2O soluble compounds or ^{14}C-starch, on a leaf area basis, does not vary significantly on these different age leaves. The results in Fig. 2 show there is 10 times more ^{14}C-assimilate in the subtending leaves than the bracts. There is about 2 to 3 times more radioactivity in the H_2O soluble compounds than starch in both leaves and bracts (see also Fig. 5).

Retention of ^{14}C-Assimilate in Subtending Leaves and Bracts. The rate of disappearance of the radioactive H_2O soluble and starch pools in leaves and bracts is shown in Fig. 3. The radioactive H_2O soluble compounds rapidly disappear out of the leaves and bracts for 6 hours. After 6 hours, there is gradual loss of ^{14}C from this pool. In contrast, the ^{14}C-starch pool remains fairly stable for 6 hours following the pulse labeling with $^{14}CO_2$. After 6 hours, the starch pool slowly disappears over the next 16 hours.

The amount of ^{14}C-assimilate export is greater than previously reported for cotton leaves (1) for a 24-hour-period. Ashley (1) found 43% of ^{14}C-assimilate synthesized in 1 hour exposure of the leaves to $^{14}CO_2$ was retained in the leaves. One of the reasons for this high percent of retention may be due to the fact that 2 hours elapsed following the pulse labeling before determining the initial amount of ^{14}C-assimilate in the leaves. The data in Fig. 3 clearly shows that the H_2O soluble ^{14}C-assimilate disappears rapidly following the exposure of the leaves to $^{14}CO_2$.

The rate of export of loss of individual components of the ^{14}C-assimilate in leaves subtending 30-day-old cotton bolls is shown in Fig. 4. The components of the H_2O soluble fraction are sugars (predominantly

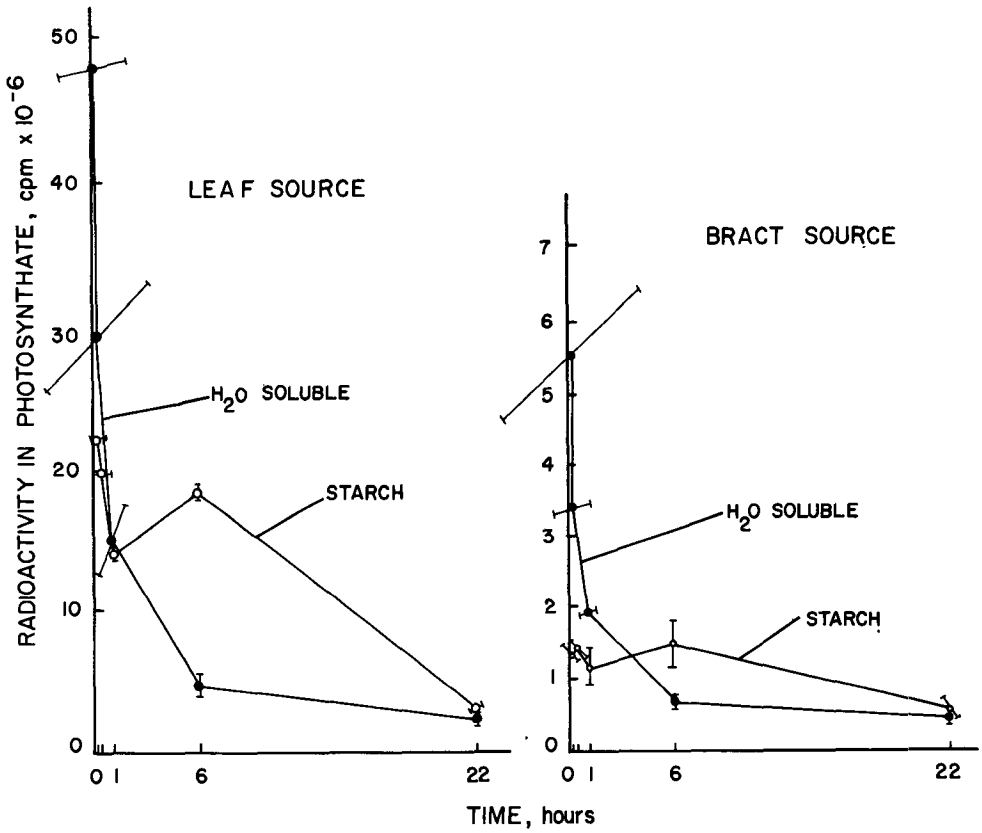

Fig. 3. Total export. Each data point is the mean of four entries and ± SD of each point is shown with a line and bar.

glucose, as shown by two-dimensional paper chromotography and autoradiography), organic acids, and amino acids. The majority of the radioactive H_2O soluble pool is ^{14}C-glucose. The rate of disappearance of ^{14}C-sugars from the leaves is nearly complete after 6 hours. There is little significant change in the radioactive organic acids in 22 hours, and there is some change in the ^{14}C-amino acids 0 to 1 hour after the pulse labeling. ^{14}C-starch is slowly exported. The amount of export of ^{14}C-starch is not as great as reported in Fig. 3.

The percent of radioactivity in the H_2O soluble and starch pools which disappears with time from the leaves and bracts is shown in Fig. 5. Following a 5 min. pulse labeling with $^{14}CO_2$, 62% of the total radioactivity fixed in the leaves and bracts is in the H_2O soluble compounds of the leaf, 28% of the total radioactivity is in leaf starch, and 10% in the H_2O soluble compounds and starch in the bracts. After 1 hour, 70% of the ^{14}C in the H_2O soluble compounds disappear from the subtending leaves. After 6 hours, over 80% of the H_2O soluble pool in both sources is exported. After 22 hours, over 90% of the ^{14}C-assimilate is exported from the leaves.

We have separately determined by acid hydrolysis (see Materials and Methods) that the amount of ^{14}C remaining in the insoluble leaf residue, following the extraction of H_2O soluble compounds and starch, is only 3 to 5% of the total $^{14}CO_2$ incorporated into the mature leaves. Clearly, the above export (Fig. 5) of the ^{14}C-assimilates can be accounted for as losses to

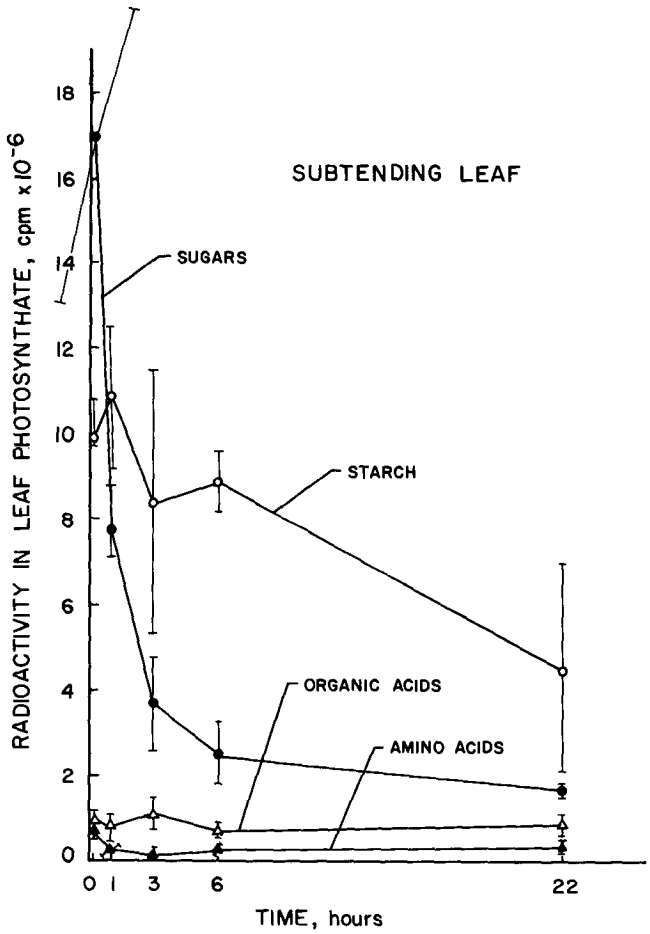

Fig. 4. Total disappearance from 30-day-old cotton bolls. The data points are the mean of two replicates and the range from each mean is shown with a line and bar.

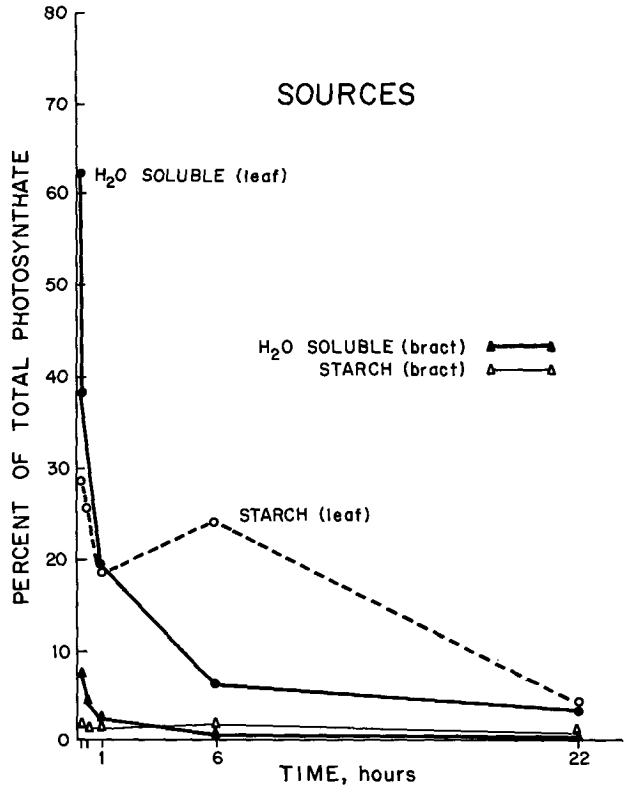

Fig. 5. Disappearance from subtending leaves and bracts.

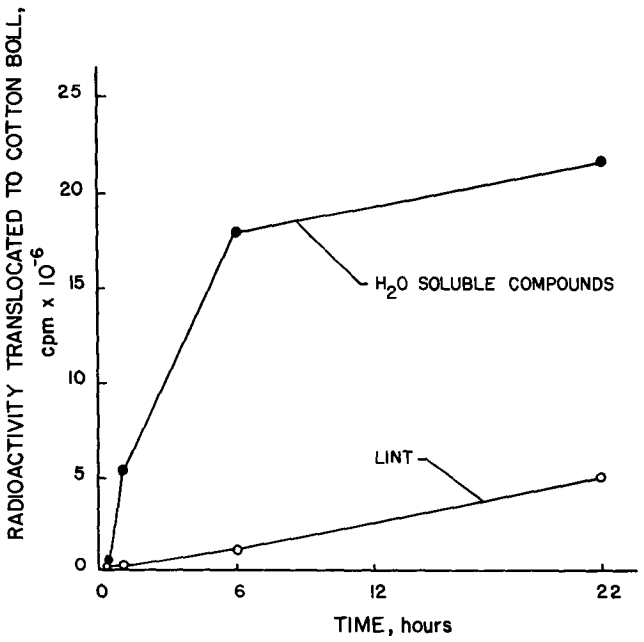

Fig. 6. Translocation from subtending leaves and bracts to bolls.

photorespiration, dark respiration, and translocation. That some of this export of ^{14}C-assimilates represents translocation to the boll sinks from the pulse labeling of leaves and branches on intact fruiting branches, is shown in Fig. 6. The transport to the cotton lint is gradual over the entire 22 hour translocation period. In contrast, the transport of the ^{14}C-assimilate to the H_2O soluble compounds in the boll wall and ovules is rapid and reaches a plateau after 6 hours. This rapid gain in ^{14}C in these boll compounds coincides with the rapid loss of ^{14}C from the H_2O soluble assimilate pool in the leaves and bracts during a 6-hour export period.

DISCUSSION

In this paper, we have studied the rate of export of ^{14}C-assimilate from different photosynthetic sources to cotton boll sinks. In a 5 min exposure to $^{14}CO_2$, leaves and bracts primarily assimilate ^{14}C into H_2O soluble compounds and starch. In a 22-hour translocation period, the leaves and bracts export the ^{14}C-assimilate to boll wall, ovules, and lint. There is no export of ^{14}C-assimilate from the cotton boll wall (capsule) to the ovules or lint. The subtending leaf is the primary photosynthetic source for the cotton boll. Compared to the subtending leaf, bracts transport only 5 to 10% of ^{14}C-assimilate to the boll constituents. As cotton bolls mature, there is no shift in the transport of ^{14}C-assimilate from the subtending leaves to the bracts or carpel wall. Morris (10) had suggested such a shift. There is however, a great difference in the rate of export and retention of H_2O soluble compounds and starch from the subtending leaves and bracts.

In other studies of the export of ^{14}C-assimilates from leaves, Hofstra and Nelson (7, 8) have shown that fully expanded leaves of C_4 plants such as corn, millet (*Setaria italica*), and sorghum (*Sorghum bicolor* L.) rapidly export ^{14}C-assimilate. About 70% of the ^{14}C-assimilate is exported in a 6-hour-period. This rapid translocation phase is followed by a slower phase over the next 18 hours. In C_3 plants such as castor bean, tobacco, and soybean, the initial translocation phase is slower. Only 40 to 50% of the assimilated ^{14}C is exported in a 6-hour-period. A comparison of the two types of translocation curves show that C_4 plants are more efficient exporters of ^{14}C-assimilate than C_3 plants. The majority of the ^{14}C-assimilate remaining in C_3 plants following a 6-hour translocation period is starch; yet little work has been done in C_3 plants comparing the rates of export of soluble sugars and starch. In this connection Thrower (12) has shown that export of assimilate in soybeans is dependent on leaf age. Hulme and Criswell (9) have exposed soybeans to $^{14}CO_2$ during a vegetative growth stage and during a rapid seed filling stage. Leaves in the upper part of the canopy during vegetative growth retain most of the ^{14}C-assimilate. Fully expanded leaves in the rapid seed filling stage export most of the ^{14}C-assimilate. At maturity, 50 to 60% of the initial ^{14}C-assimilate can be recovered in the seed.

In cotton plants, following a 5 min pulse with $^{14}CO_2$, there is an initial rapid export phase of radioactive H_2O soluble compounds from the photosynthetic sources. After 6 hours, over 80% of the H_2O soluble pool in both leaf and bract sources is exported. After 22 hours, over 90% of this pool disappears. In contrast, there is a very slow export of radioactive starch up to 6 hours after exposing the plants to $^{14}CO_2$. After 6 hours, there is a steady decline in ^{14}C-starch. At the end of a 22-hour translocation period, about 90 to 95% of the ^{14}C-assimilate is exported from the mature subtending leaves and associated bracts. We have shown that a portion of the ^{14}C-assimilate export represents translocation to the boll sinks. The transport of ^{14}C to H_2O soluble compounds in the boll wall and ovules coincides with the loss of ^{14}C from the H_2O soluble pool in the leaves and bracts in a 6-hour translocation period.

We conclude that the export of H_2O soluble assimilate from the leaves and bracts of cotton (a C_3 plant) is similar to the export of ^{14}C-assimilate in C_4 plants. Compared to C_4 plants, cotton leaves and bracts retain a greater amount of assimilate as starch and export this slowly.

REFERENCES

1. Ashley, D. A. 1972. ^{14}C-Labeled photosynthate translocation and utilization in cotton plants. Crop Sci. 12:69-74.
2. Benedict, C. R., K. J. McCree, and R. J. Kohel. 1971. High photosynthetic rate of a chlorophyll mutant of cotton. Plant Physiol. 49:968-971.
3. Benedict, C. R., R. H. Smith, and R. J. Kohel. 1973. Incorporation of ^{14}C-photosynthate into developing cotton bolls. Crop Sci. 13:88-91.
4. Brown, K. J. 1968. Translocation of carbohydrate in cotton: Movement to the fruiting bodies. Ann. Bot. 32:703-713.
5. ———. 1973. Factors affecting translocation of carbohydrate to fruiting bodies of cotton. Cotton Grow. Rev. 50:32-42.
6. Hartt, C. E., and H. P. Kortschak. 1967. Translocation of ^{14}C in the sugarcane plant during the day and night. Plant Physiol. 42:89-94.
7. Hofstra, G., and C. D. Nelson. 1969. The translocation of photosynthetically assimilated ^{14}C in corn. Can. J. Bot. 47: 1,435-1,442.
8. ———, and ———. 1969. A comparative study of translocation of assimilated ^{14}C from leaves of different species. Planta (Berlin) 88:103-112.
9. Hulme, D. J., and J. G. Criswell. 1973. Distribution and utilization of ^{14}C-labeled assimilates in soybeans. Crop Sci. 13:519-524.
10. Morris, D. A. 1964. Photosynthesis by the capsule wall and bracteoles of the cotton plant. Emp. Cotton Grow. Rev. 41:49-51.
11. Muramoto, H., J. D. Hesketh, and C. D. Elmore. 1967. Leaf growth, leaf aging and leaf photosynthetic rates of cotton plants. Beltwide Cotton Prod. Res. Conf., Proc. (Dallas) p. 161-165.
12. Thrower, S. L. 1962. Translocation of labeled assimilates in the soybean. II. The pattern of translocation in intact and defoliated plants. Aust. J. Biol. Sci. 15:629-649.