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Evidence for Light-Dependent Recycling of Respired Carbon Dioxide by the Cotton Fruit[1]

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ABSTRACT

Conservation of respired CO_2 by an efficient recycling mechanism in fruit could provide a significant source of C for yield productivity. However, the extent to which such a mechanism operates in cotton (*Gossypium hirsutum* L.) is unknown. Therefore, a combination of CO_2 exchange, stable C isotope, and chlorophyll (Chl) fluorescence techniques were used to examine the recycling of respired CO_2 in cotton fruit. Respiratory CO_2 losses of illuminated fruit were reduced 15 to 20% compared with losses for dark-incubated fruit. This light-dependent reduction in CO_2 efflux occurred almost exclusively via the fruit's outer capsule wall. Compared with the photosynthetic activity of leaves, CO_2 recycling by the outer capsule wall was 35 to 40% as efficient. Calculation of $^{14}CO_2$ fixation on a per Chl basis revealed that the rate of CO_2 recycling for the capsule wall was 62.2 micromoles $^{14}CO_2$ per millimole Chl per second compared with an assimilation rate of 64.6 micromoles $^{14}CO_2$ per millimole Chl per second for leaves. During fruit development, CO_2 recycling contributed more than 10% of that C necessary for fruit dry weight growth. Carbon isotope analyses ($\delta^{13}C$) showed significant differences among the organs examined, but the observed isotopic compositions were consistent with a C_3 pathway of photosynthesis. Pulse-modulated Chl fluorescence indicated that leaves and fruit were equally efficient in photochemical and nonphotochemical dissipation of light energy. These studies demonstrated that the cotton fruit possesses a highly efficient, light-dependent CO_2 recovery mechanism that aids in the net retention of plant C and, therein, contributes to yield productivity.

Direct C assimilation by fruit and the subsequent availability of this photosynthate for yield productivity has generally been regarded as insignificant compared with that photosynthate supplied by leaves (5, 21, 24). However, although dehiscent and indehiscent fruit often exhibit negligible fixation of atmospheric CO_2, these organs nonetheless frequently demonstrate a unique capacity for the reassimilation of internally produced CO_2, especially that CO_2 released via mitochondrial respiration from developing ovules (4, 9). Cereal crops have received the greatest attention in this regard (5, 14, 25), although several studies have dealt with other crops as well (2, 11, 15, 16, 23). The efficiency with which fruit are able to conserve respired C for subsequent retranslocation back to the ovule could be an important determinant of yield productivity (11).

Mechanisms that govern the recycling of CO_2 released via fruit respiration were investigated by Kriedemann (14) who observed a light requirement for the reassimilation process in whole ears of wheat (*Triticum vulgare* L.). Watson and Duffus (22) recently extended these studies by labeling caryopses of barley (*Hordeum vulgare* L.) with $^{14}CO_2$ and showed that as much as three times more ^{14}C was retained by the caryopses after incubation in the light compared with dark-incubated caryopses. These authors concluded that the pericarp functioned as an efficient tissue for the reassimilation of $^{14}CO_2$ respired by the endosperm. Similar studies have also shown the pod wall of pea (*Pisum sativum* L.) to contain two distinct photosynthetic layers, each capable of contributing photosynthate to seed development (2). The outer pod wall fixed CO_2 from the ambient atmosphere, and the chloroplast-containing inner wall was involved in the photoassimilation of CO_2 released from seed respiration. Crookston *et al.* (7) indicated that recycling of internally released CO_2 (*i.e.* CO_2-fixing potential) by the pod wall of *Phaseolus vulgaris* L. was substantial and estimated its photosynthetic capacity to be >25% that of the leaf.

Kriedemann (14) proposed that under natural conditions the effective recycling of respired CO_2 could make a significant contribution to yield by reducing respiratory C losses. We previously documented that diurnal C losses from cotton (*Gossypium hirsutum* L.) fruit can exceed 55% of the maximum daily C gain of these organs (24) and provided preliminary data concerning a light-dependent mechanism for CO_2 reassimilation by the capsule wall (23). The efficiency by which this CO_2 recycling offsets respiratory C losses could be an important aspect related to crop productivity. Therefore, the objectives of this study were to verify the light dependency of CO_2 recycling by cotton fruit, document the efficiency by which this mechanism operates, and evaluate the contribution of CO_2 recycling to the stable C isotope composition of the cotton fruit.

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MATERIALS AND METHODS

Field Crop Management

Seeds of cotton (*Gossypium hirsutum* L., cv Stoneville 506) were planted into a Captina silt loam soil (Typic Fragiudult) on May 24, 1990, at the University of Arkansas, Agricultural Experiment Station, Fayetteville, AR. Plots consisted of eight rows, spaced 1 m apart, and were thinned to 77,000 plants ha^{-1} after the stands were established. Fertilizer consisted of a preplant application of 37–16–30 kg ha^{-1} of N-P-K followed by two midseason side-dressings of 30 kg N ha^{-1} as ammonium nitrate at 8 and 11 weeks. Furrow irrigation provided a well-water environment (*i.e.* midday leaf water potentials >-1.8 MPa), and insecticides were applied as needed during the season.

Light Dependency of CO_2 Recycling

Experiment I

Differences in CO_2 evolution between light- and dark-incubated cotton fruit were used to initially investigate the light dependency of CO_2 recycling. Twenty-d-old fruit (*i.e.* 20 d after anthesis) were excised from field-grown plants and placed into an 0.25-L stirred cuvette, and CO_2 exchange rates were monitored with a model LI-6000 photosynthesis system (Li-Cor Inc., Lincoln, NE). Measurements were taken for 30 s under ambient irradiance (PAR >1500 μmol m^{-2} s^{-1}) and then repeated a second time after covering the cuvette with a black cloth. The cuvette was ventilated with ambient atmosphere following imposition of the dark treatment for 60 s before beginning data acquisition. Light-induced reductions in CO_2 evolution from fruit were taken to broadly indicate CO_2 recycling. However, we acknowledge that this procedure may overestimate CO_2 recycling, particularly if light inhibits mitochondrial CO_2 efflux (*i.e.* dark respiration).

Experiment II

The light-dependent capacity of cotton fruit to assimilate respired CO_2 was further investigated by injecting $^{14}CO_2$ into 20-d-old fruit. Radiolabeled CO_2 was generated by adding 4 mL of 2.5 M lactic acid to a scintillation vial containing 1.85×10^5 Bq of $NaH^{14}CO_3$ (specific activity 3.52×10^9 Bq $mmol^{-1}$). The vial was enclosed within a 1000-mL side-arm flask fitted with a rubber septum. A glass syringe equipped with a 22-gauge needle was used to withdraw a 0.1-cm^3 sample of $^{14}CO_2$ for injection into each of the four cotton fruit locules. Excised fruit were then immediately sealed within 300-cm^3 glass jars and randomly assigned to one of six shade treatments. Each glass jar also contained a vial filled with 3 mL of Carbo-Trap 2 (JT Baker Co., Phillipsburg, NJ) to trap $^{14}CO_2$ respired by the fruit. Shading was achieved by using a series of shade cloths, either alone or in combination, to simulate a 32, 47, 69, 83, and 100% reduction in ambient irradiance. Experiments were terminated after 60 min and temperature fluctuations were minimized during this time by placing the jars on a shallow bed of ice. Sample ^{14}C activity of the trapping solution was counted with a Packard Tri-Carb 4530 liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL).

Experiment III

The efficiency with which fruit (*i.e.* the capsule wall) assimilated $^{14}CO_2$ was compared with that of other cotton organs. Ten sample discs (1 cm diameter) were removed from leaves, bracts, and capsule walls (Fig. 1) and placed on moistened filter paper within a plastic container (58 × 43 × 15 cm) equipped with a glass lid and a circulation fan. Leaf and bract samples were oriented with their adaxial surface facing up. Two vials filled with 3.70×10^5 Bq $NaH^{14}CO_3$ (specific activity 3.52×10^9 Bq $mmol^{-1}$) were also enclosed within the above-mentioned plastic container. Evolution of $^{14}CO_2$ was initiated after the addition of 3 mL lactic acid to each vial. Sample assimilation of $^{14}CO_2$ took place for 10 min, after which the container was quickly evacuated to trap excess $^{14}CO_2$ in a soda lime cartridge. Samples were then placed in test tubes containing 9 mL cold 80% ethanol, packed in ice, and immediately transferred to the laboratory. Tissues were homogenized and centrifuged at 3000 rpm for 15 min, and hydrogen peroxide was added to the supernatant for a final concentration of 3%. Extracts were photobleached at an elevated irradiance overnight. Subsamples (1 mL) of the extracts were added to 12 mL ScintiVerse E and analyzed with a Packard Tri-Carb scintillation spectrometer.

Stable Carbon Isotope Composition

Stable carbon isotope abundances were determined for several vegetative and reproductive organs selected from the first fruiting position of main stem node 8 at 30 d after anthesis. Replicate samples were pooled, oven dried at 70°C, and finely ground to pass a 40-mesh screen. Subsamples were combusted, and the relative abundance of ^{13}C and ^{12}C in the CO_2 produced was analyzed by mass spectrometry using the isotope ratio mass spectrometer facilities administered by the Department of Biology, University of Utah. Stable carbon isotope composition was expressed as the $^{13}C/^{12}C$ ratio relative

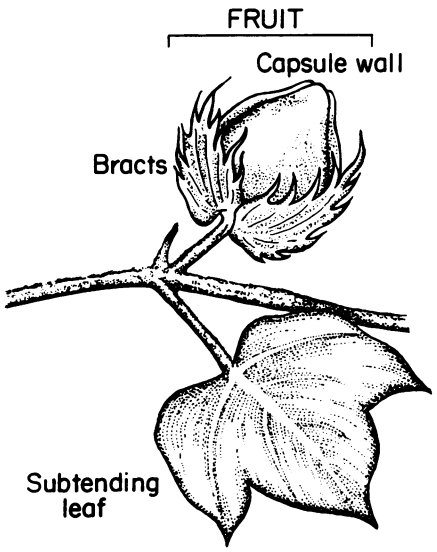

Figure 1. Spatial relationship of the subtending leaf, bracts, and the capsule wall.

Table I. *Net CO_2 Efflux from Cotton Fruit as Affected by the Presence or Absence of Irradiance*

Fruit Age	Treatment		Apparent Recycling
	Light	Dark	
d	*μmol m^{-2} s^{-1}*		%
1–10	32.5 ± 1.3[a]	32.7 ± 1.8 NS[b]	
11–20	16.4 ± 1.4	19.2 ± 1.7*	14.6
21–30	8.2 ± 0.7	10.3 ± 1.0*	20.4
31–40	8.3 ± 1.1	9.7 ± 1.3*	14.5

[a] Mean ± SD (n = 8). [b] Differences between treatment means are indicated either NS or * (significant at $P < 0.05$).

Table II. *Net Exchange Rates of $^{14}CO_2$ by Excised Discs of Cotton Leaves, Bracts, and the Capsule Wall*

Organ	Surface	Treatment	
		Light	Dark
		μmol $^{14}CO_2$ m^{-2} s^{-1}	
Leaves	Adaxial	25.4 ± 5.9[a] a[b]	1.2 ± 0.5 b
Bracts	Adaxial	3.6 ± 1.7 c	1.2 ± 0.5 b
Capsule wall	Intact	10.4 ± 1.9 b	2.9 ± 0.4 a
	Outer wall	10.7 ± 1.2 b	2.4 ± 0.6 a
	Inner wall	0.7 ± 0.2 (NS)	0.2 ± 0.1 (NS)

[a] Mean ± SD (n = 4). [b] Means followed by the same letter within a column are not significantly different at $P < 0.05$.

to that of the Pee Dee belemnite standard (6). The analyses were repeated in triplicate.

Chl Fluorescence and Pigment Analysis

Fluorescence induction curves were measured for dark-adapted (30 min) leaves, bracts, and fruit that were collected before sunrise from a mature cotton canopy. Fluorescence parameters were determined by the saturated pulse method (18, 20) using a PAM 101 Chl fluorometer (H. Walz, Effeltrich, Germany). The fluorescence measuring beam (0.1 μmol photons m^{-2} s^{-1}, modulated at 1.6 kHz), saturating pulse (2550 μmol photons m^{-2} s^{-1}, 800 ms), and red actinic irradiance (50 μmol photons m^{-2} s^{-1}) were delivered to the adaxial surface of leaves and bracts, or the capsule wall of fruit, via fiberoptics.

Two components of fluorescence quenching were recorded using the equations of Schreiber *et al.* (18):

$$q_Q = \frac{(F_v)_s - F_v}{(F_v)_s} \text{ and } q_E = \frac{(F_v)_m - (F_v)_s}{(F_v)_m} \quad (1)$$

where F_v = variable fluorescence, $(F_v)_m$ = maximal variable fluorescence, and $(F_v)_s$ = time-dependent variable fluorescence. Photochemical quenching (q_Q[2]) was used to indicate the relative oxidation state for the primary acceptor (Q_A) in PSII, whereas nonphotochemical quenching (q_E) was interpreted as a measure of the proton gradient across the thylakoid membrane and, hence, an indicator of the ATP-generating capacity of the selected organ.

Chl was extracted from fresh tissue by homogenization in 20 mL cold 80% acetone (v/v). After centrifugation (1500 rpm, 5 min) at room temperature, absorbance of the supernatant was measured at 645 and 663 nm on a spectrometer (Hewlett-Packard Co., Cupertino, CA), and Chl content was calculated using the formula of Arnon (1).

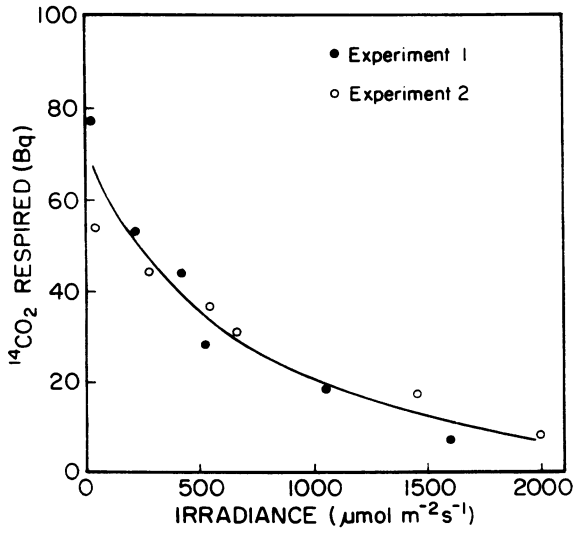

Figure 2. Respiration of $^{14}CO_2$ from cotton fruit as a function of irradiance. Each point represents the mean of three replicates.

RESULTS AND DISCUSSION

Differences in the CO_2 exchange rate of cotton fruit exposed to either light or dark conditions were observed with decreased evolution of CO_2 from light-treated organs (Table I). Although not significant for young fruit, CO_2 losses from illuminated fruit were reduced 15 to 20% compared with losses from dark-incubated fruit. The magnitude of this apparent CO_2 recycling was strongly dependent on irradiance and varied in a curvilinear manner between the two light/dark extremes (Fig. 2). Progressive increases in irradiance up to 2000 μmol PAR m^{-2} s^{-1} steadily reduced the efflux of $^{14}CO_2$ from 20-d-old fruit by more than fourfold.

Assimilation of CO_2 by the fruit's capsule wall was considerable compared with the photosynthetic activity of other organs within the crop canopy (Table II). When exposed to ambient irradiance, the capsule wall assimilated $^{14}CO_2$ at a rate of >10.0 μmol m^{-2} s^{-1}. The majority of this assimilation occurred via the fruit's outer cell layers with little $^{14}CO_2$ fixation by the inner capsule wall. Compared with the photosynthetic activity of leaves, CO_2 recycling by the outer capsule wall was 35 to 40% as efficient. Bracts were also photosynthetically active, although $^{14}CO_2$ assimilation rates for these organs were fourfold lower than those of the capsule wall and only 10% those of leaves (Table II). Organ-specific

[2] Abbreviations: q_Q, coefficient of photochemical quenching; Q_A, primary electron acceptor of PSII; q_E, coefficient of nonphotochemical quenching; $\delta^{13}C$, stable carbon isotope composition.

differences in dark $^{14}CO_2$ assimilation were also observed between leaves, bracts, and the capsule wall with the outer capsule wall apparently capable of light-independent $^{14}CO_2$ fixation.

Blanke and Lenz (4) summarized recent literature regarding fruit gas exchange and concluded that fruit photosynthesis often compensates for respiratory CO_2 losses. Whereas this balance of CO_2 fluxes may occur in certain legume and grain crops, previous studies have indicated that cotton fruit do not assimilate CO_2 from the ambient atmosphere (8, 16, 23). This lack of CO_2 assimilation was recently shown by Wullschleger and Oosterhuis (23) to be related not to an impaired photosynthetic capacity but rather to an overwhelmingly large CO_2 gradient from the fruit interior (0.45–1.53% CO_2, v/v) to the external atmosphere. Black and Vines (3) proposed that, even when net fixation of atmospheric CO_2 does not occur in fruit, these organs can exhibit effective internal CO_2 retrieval cycles and, therein, contribute to the net retention of plant C.

Cotton fruit in our study was shown to possess a highly efficient recycling mechanism and, therefore, the extent to which such a mechanism operates could be a significant means of offsetting respiratory CO_2 losses. Our results indicated that light-dependent recycling of CO_2 reduced fruit respiration by 2.1 μmol m^{-2} s^{-1} which, for 21 to 30-d-old fruit, would account for an average retention of 15 mg C $fruit^{-1}$ d^{-1}. Throughout the duration of fruit development (>40 d), this retention of C could approximate 10% of that necessary for fruit dry weight growth. This observation might explain why shading of the capsule wall can lead to reduced fruit growth (16). Flinn *et al.* (9) observed similar findings in pea and emphasized that, because of the light dependency of CO_2 recycling, attention should be given to achieve adequate irradiance of legume fruits when designing agronomic practices or when breeding for more desirable canopy architecture.

Differences in the assimilatory capacity of leaves, bracts, and the capsule wall were evaluated with respect to light-harvesting pigments and the photochemical potential of these organs to utilize light energy for CO_2 reduction. Leaves contained the greatest amount of total Chl (Chl a + b) with concentrations exceeding 425 μmol m^{-2} followed by Chl concentrations of the capsule wall and bracts, which averaged 48 and 35%, respectively, that of the leaves (Table III). The majority of Chl in the capsule wall (>80%) was contained within the outer layer. Chl a/b ratios were greatest for the leaves but were similar for bracts, capsule wall (intact), and outer capsule wall (Table III).

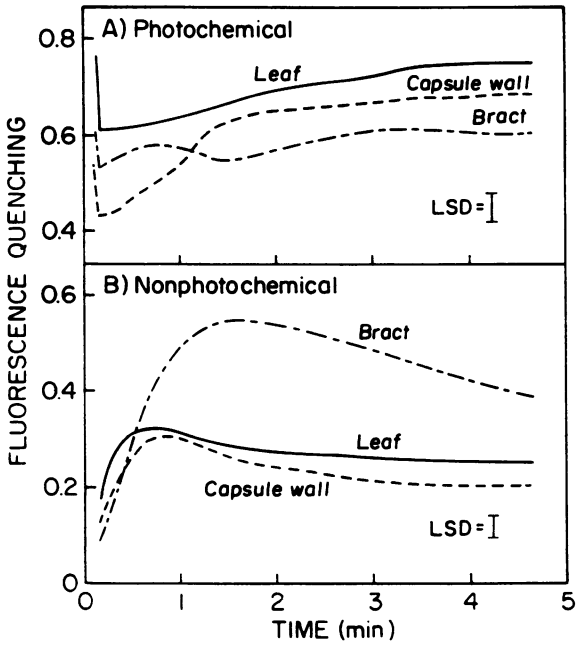

Figure 3. Photochemical (A) and nonphotochemical (B) quenching by cotton leaves, bracts, and the capsule wall as determined by pulse-modulated fluorescence techniques. Curves represent the mean of four replicates.

The overwhelming presence of Chl in the outer capsule wall of cotton fruit was consistent with the predominant assimilatory capacity of these tissues for light-dependent $^{14}CO_2$ fixation. Although $^{14}CO_2$ assimilation by the capsule wall was less than that of the leaves, recalculation of $^{14}CO_2$ fixation on a per Chl basis revealed that the rate of CO_2 recycling for the capsule wall was 62.2 μmol $^{14}CO_2$ $mmol^{-1}$ Chl s^{-1} compared with an assimilation rate of 64.6 μmol $^{14}CO_2$ $mmol^{-1}$ Chl s^{-1} for leaves at comparable levels of irradiance. Sestak and Catsky (19; see also ref. 4) observed similar results in that the photosynthetic rates of apple fruit, when calculated on a Chl basis, were also commensurate with those of leaves.

Fluorescence induction curves for leaves, bracts, and the capsule wall indicated organ-specific differences in light energy dissipation or quenching (Fig. 3). Photochemical quenching (q_Q) for all organs decreased initially with the onset of actinic radiation and then gradually increased, indicating a

Table III. *Chl Concentrations of Cotton Leaves, Bracts, and the Capsule Wall*

Tissue	Chl a	Chl b	Total	Chl a/b
		μmol Chl m^{-2}		
Leaves	306 ± 15[a] a[b]	123 ± 15 a	429 ± 29 a	2.48 ± 0.20 a
Bracts	102 ± 10 c	47 ± 8 b	149 ± 18 c	2.17 ± 0.19 b
Capsule wall				
Intact	141 ± 18 b	67 ± 10 b	208 ± 28 b	2.10 ± 0.05 b
Outer wall	118 ± 22 bc	54 ± 10 b	172 ± 31 bc	2.19 ± 0.15 b
Inner wall	22 ± 4 d	13 ± 4 c	35 ± 8 d	1.69 ± 0.06 c

[a] Mean ± SD (n = 6). [b] Means followed by the same letter within a column are not significantly different at $P < 0.05$.

reoxidation of the primary electron acceptor (Q_A) of PSII (Fig. 3A). Leaves and capsule walls generally exhibited similar trends in photochemical quenching, whereas the low (or slowly increasing) q_Q of the bracts indicated that Q_A remained in a slightly more reduced state (18). In comparison, the induction of nonphotochemical quenching (q_E) for leaves and the capsule wall also paralleled one another, in contrast to the marked increase in q_E for the bracts (Fig. 3B). Elevated q_E as characterized by the bracts is indicative of an increase in the transmembrane proton gradient of the thylakoids and a reduced ATP turnover, possibly arising from a limitation in CO_2 assimilation (13).

Chl fluorescence techniques have not, to our knowledge, been used to investigate light-dependent processes in fruit. Based on our experience, albeit limited, the use of pulse-modulated fluorescence provides a valuable tool for combining aspects of fruit gas exchange, and in particular light-energy utilization in CO_2 recycling, together with electron transfer through the photosystems and ultimately to CO_2. Recently, Stuhlfauth *et al.* (20) presented gas exchange information complemented with Chl fluorescence data to suggest that reassimilation of internally evolved CO_2 from water-stressed leaves was an effective mechanism for the dissipation of excess light energy in *Digitalis lanata*. Light energy dissipation by fruit in the form of an effective CO_2-recycling mechanism has not been addressed but could play a central role in protecting these sensitive tissues from photooxidation. The importance of CO_2 recycling in fruit as a mechanism of light energy dissipation awaits further investigation.

Hubick and Farquhar (12) suggested that fractionation of plant carbon may occur during respiration resulting in a less negative $\delta^{13}C$ for respired CO_2 than corresponding data for atmospheric CO_2. Thus, if CO_2 recycling contributes to the C economy of an organ, then presumably such tissues should show $\delta^{13}C$ values less negative than those of translocated carbon (*i.e.*, leaf $\delta^{13}C$). We observed that for a range of organs, $\delta^{13}C$ values were within that normally predicted from C contributed by C_3 photosynthesis (Table IV). Although these $\delta^{13}C$ values were sometimes significantly different, *e.g.* between leaves and the corolla, they did not suggest substantial CO_2 fixation via a pathway other than that typical of C_3 plants. However, it is difficult to assess these results because different plant organs have differing proportions of various compounds (*i.e.* lipids, cellulose) which are often differentially labeled with respect to the C isotopes (12, 17). Hall *et al.* (10) reported that ^{13}C isotope discrimination for grains of cowpea [*Vigna unguiculata* (L.) Walp.] was lower than that measured for subtending leaves and suggested that additional metabolic processes were responsible for the lower ^{13}C discrimination in grains.

Table IV. *$\delta^{13}C$ of Selected Organs from the Cotton Canopy*

Tissue	Year	
	1989	1990
	‰	
Leaves	−27.2 b[a]	−26.4 c
Bracts	−29.1 c	−26.9 cd
Capsule wall		
Intact	−26.2 a	NA[b]
Outer wall	NA	−27.7 e
Inner wall	NA	−27.3 de
Corolla	NA	−24.9 a
Fiber	−26.3 a	NA
Seed	−25.9 a	NA
Stem	NA	−26.5 b

[a] Means followed by the same letter within a column are not significantly different at $P < 0.05$. [b] NA = not available.

In summary, cotton fruit possessed an efficient, light-dependent mechanism for the recovery of respired CO_2 which contributed significantly to the net retention of plant C for yield productivity. This recycling of CO_2 by the fruit's outer capsule wall was 35 to 40% of the photosynthetic activity of leaves, and both leaves and fruit exhibited equally efficient patterns of light energy dissipation. The extent to which photochemical quenching by the fruit and CO_2 recycling are related awaits further study. However, it is suggested that CO_2 recycling may aid in the dissipation of excess light energy that might otherwise contribute to the photooxidation of these sensitive structures.

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