GROWTH OF COTTON LEAVES AND BRACTS AND THEIR CARBON CONTRIBUTION TO DEVELOPING SQUARES

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Abstract

Development of cotton (*Gossypium hirsutum* L.) squares is fundamental for flowering, fruiting and yield formation. However, little is known about the carbon translocated to developing floral buds from leaves and floral bracts. Studies were conducted under field and greenhouse conditions to characterize the morphology and physiology of square development, and to quantify carbon contribution of leaves and bracts to a floral bud. During square ontogeny, increases in area and dry weight (DW) of floral bracts, as well as the subtending sympodial leaf, followed a sigmoid growth curve with increasing square age. Net photosynthetic rate (P_n) of the sympodial leaf at the first fruiting branch position of main-stem node 10 reached a maximum when the subtended square developed into a white flower. Floral bracts had much lower P_n and higher dark respiration rate than the subtending sympodial leaf. Total nonstructural carbohydrate concentrations in the bracts were also lower than that in the leaves during square development. Bracts also had a higher sucrose fraction of total nonstructural carbohydrates than the leaves. The amount of $^{14}CO_2$ fixation by the bracts of a 20-day-old square was only 22% of the subtending leaf. However, at 6 hr after ^{14}C feeding these sources, the bracts had supplied 56% of ^{14}C-assimilate to the floral bud, and only 27% from the subtending leaf and 17% from the main-stem leaf. These results indicated that floral bracts might play an important role in the carbon supply of developing cotton squares because bract ^{14}C-photoassimilates moving into the subtended floral buds was much more rapid than that of the leaves.

Introduction

The formation and development of cotton squares are fundamental for yield. However, squares often fail to form flowers (Guinn, 1982). If failure occurs with too many squares abscised, maturity can be delayed and lint yield can be reduced. Therefore, a better understanding of initiation, differentiation and development of squares, and the characteristics of their physiology and biochemistry during development is important for improving cotton production.

Most previous studies on cotton fruit development and their contributory photoassimilate sources have focused on the developing bolls. However, carbon contribution of different sources to square development has not been clearly documented. Our hypothesis was that bracts may play a more important role in providing assimilate for square development than for boll development, because the subtending sympodial leaf only unfolded 7 to 10 days after the square first appeared (Zhao, 1997). The objectives of the present studies were (1) to quantify the changes in area and dry weight (DW) of the subtending leaf and bracts, bud DW, and net photosynthetic rate (P_n) of the leaves and floral bracts during square development, and (2) to quantify the carbon contribution of leaves and bracts to square growth.

Materials and Methods

Field Studies

The cotton cultivar Deltapine 20 was machine-planted in the field at the Arkansas Agricultural Research and Extension Center, University of Arkansas in Fayetteville, AR, on 4 June 1993 , 17 May 1994 and 19 May 1997. Rows were spaced 1 m apart and oriented in a north-south direction. Plants were hand-thinned to a density of 9 plants m^{-1} row when the seedlings had three true leaves. Fertilizer, control of weeds and insects, and furrow irrigation were applied as needed during the growing seasons according to Arkansas cotton extension recommendations. The field was divided into two blocks for sampling and for determining final boll economical properties and lint yield. Each block consisted of three plots (replications). The plot size was 10 × 15 m (1993 and 1994) or 5 × 5 m (1997).

When the squares at the fruiting position 1 of main-stem node (MSN) 10 first became visible (about 3 mm in diameter), they were individually labeled by tagging main-stem leaves at the respective node with dated jeweler's tags. Approximately 900 (1993 and 1994) or 100 (1997) squares similar in size at this position from three replications were tagged in a day. Squares at this date were considered day zero in age. During development of the tagged squares in 1993 and 1994, samples of the squares and the sympodial leaves subtending the squares were taken at 5, 10, 15, 20 and 25 days after the tagging date until the squares developed into white flowers (anthesis). Up to 30 tagged squares and subtending sympodial leaves in each plot of three replications were randomly collected starting at 9:00 a.m. at each sampling date. The tissues were immediately transported to the laboratory. Squares were separated into floral buds and bracts. Areas of leaves and bracts were measured individually. Thereafter, tissues were dried at 90 ^oC for 30 min and followed by 24 h at 70 ^oC in a forced draft oven, and weighed. The dried tissues were ground and passed through a 0.5 mm sieve to determine nonstructural carbohydrate concentrations in these tissues (Zhao and Oosterhuis, 1998).

Greenhouse Study

Cotton (cv. Deltapine 20) seeds were planted in four 0.3-m^3 polyvinylchloride pots filled with Captina silt loam from a

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cotton field on 21 April 1995. After emergence, seedlings were thinned to two plants per pot. Water and fertilizer were supplied as needed and the greenhouse temperature during growth ranged between 30 °C (day) and 20 °C (night).

From the day of first square appearance (3 mm in diameter), all visible squares at all fruiting positions were labeled daily with dated jeweler's tags. When the first square on the plant became a white flower, the P_n and dark respiration rates of all tagged squares, which included the bracts and floral bud, and sympodial leaves subtending the squares in the plant canopy were individually determined with a LI-6200 photosynthesis system and a custom built cylindrical cuvette with a volume of 0.325 L. Thereafter, the bud was removed carefully from the square with a razor blade, and the cut was immediately sealed with Vaseline. The dark respiration of bracts alone was then measured. When measuring the dark respiration rate, the cuvette was covered with a double-layer of aluminum foil painted inside with black paint. Bud dark respiration and bract P_n were calculated by subtracting bract dark respiration from dark respiration of a square (bud + bracts), and subtracting bud respiration from square P_n, respectively.

$^{14}CO_2$ Fixation and ^{14}C-Assimilate Translocation Study

In 1997, when tagged squares at the fruiting position 1 of MSN 10 were 20 days in age, carbon fixation and translocation of the main-stem leaf, the sympodial leaf at fruiting position 1 and bracts of tagged squares at MSN 10 were measured by monitoring the ^{14}C radioactivity in various plant organs following exposure of selected leaves or bracts to $^{14}CO_2$. The three $^{14}CO_2$-feeding treatments were (1) the main-stem leaf at MSN 10, (2) the sympodial leaf at fruiting position 1 of MSN 10, and (3) the bracts of the square at fruiting position 1 of MSN 10 (Fig. 1). Each treatment included 6 plants from 3 replications.

The ^{14}C-labeling technique utilized was similar to that of Wullschleger and Oosterhuis (1990). The main-stem leaf, sympodial leaf and floral bracts at fruiting position 1 of MSN 10 were individually exposed to $^{14}CO_2$ for 15 min starting at 11:30 a.m.. Tissues of the petiole and blade of the source leaf, and the peduncle, bracts and floral bud of the tagged square were collected 6 h after $^{14}CO_2$-feeding. Samples were dried at 70 °C for 72 h in a forced draft oven, and weighed. Individual samples were subsequently combusted in a sample oxidizer, and the ^{14}C radioactivity counted in a Packard Tri-Carb 4530 liquid scintillation spectrometer.

Results and Discussion

Growth of a Square and Subtending Sympodial Leaf

During square development, the increases in the area and DW of bracts of a square exhibited a typical sigmoid growth curve with increasing square age, and reached near maximum values when the square became a white flower (Fig. 2). The period of maximum growth rates of bract area enlargement and DW accumulation was between 14 and 18 days after the square was first visible (about 3 mm in diameter).

The increase in the DW of a floral bud with increasing square age showed a typically exponential growth curve (Fig. 2B). Bud DW increased slowly during the first 15 days of square development. Thereafter, the DW increased rapidly, and tripled at one day before anthesis compared to the DW at 15 days in age.

Patterns of changes in LA and DW of the sympodial leaf subtending a square with increasing square age were similar to those of the floral bud bracts (Fig. 2). The sympodial leaf and floral bracts at a specific fruiting position had similar area and DW within the first 10 days of the square development. Thereafter, the subtending sympodial leaf exhibited significantly greater LA and DW than bracts of the square. The LA and DW of the sympodial leaf were 3 and 5 folds greater than those of floral bracts, respectively, when the square became a white flower.

Gas Exchange Characteristics of Leaves and Bracts

Under greenhouse conditions, P_n of bracts ranged -2.0 to 2.0 μmol m^{-2} s^{-1} during square ontogeny, and showed no clear pattern of change with square age (Fig. 3). Compared to leaf photosynthetic properties, bract P_n was very low, and the change in bract P_n with square age was less than that in leaf P_n. During square development, dark respiration rate of bracts for 10- to 15-day-old squares was highest, and decreased with increasing square age. In contrast, the dark respiration of the sympodial leaf subtending a square was much lower than that of the bracts, and showed much less change with square age.

The P_n of bracts during boll development has been studied by several researchers using different methods (Ashley, 1972; Benedict et al., 1973; Wullschleger and Oosterhuis, 1990; Bondada et al., 1993). All of these researchers found that the contribution of bract photosynthesis to boll development was much smaller than the leaves. Our greenhouse study indicated that P_n of bracts during square ontogeny was only 10 to 15% of the subtending leaf P_n (Fig. 4). Bondada et al. (1993) pointed out that low P_n of bracts was probably associated with a poor anatomical structure and light conditions because bracts had significantly lower stomatal density and lower chlorophyll concentration than leaves. However, in our $^{14}CO_2$ fixation study, we found that ^{14}C radioactivity per unit DW was similar between bracts of 20-day-old squares and the subtending leaf (Table 1). During square development, the specific bract weight (5.2 mg cm^{-2}), averaged cross sampling dates and years, was much smaller than the specific leaf weight of the subtending leaf (9.9 mg cm^{-2}). Since the P_n is usually expressed on a LA basis or on bract area basis, lower bract P_n was also related to a smaller bract DW:area ratio compared to the leaf. Generally, carbon contribution of bracts to fruit was

smaller than the subtending leaf because the area and DW of the bracts of a square were only about 36% and 19% of the subtending leaf, respectively, at flowering (Fig. 2). Additionally, low bract P_n was also associated with a higher bract dark respiration rate compared to the leaf (Fig. 3).

Nonstructural Carbohydrate Concentrations in Leaves, Bracts and Floral Buds

The patterns of changes in nonstructural carbohydrate concentrations of leaves, bracts, and floral buds during square development are shown Fig. 4. In the three tissues measured, starch was a dominant nonstructural carbohydrate which accounted for 65-80% of total nonstructural carbohydrate (TNC) concentration. Of the three tissues, bracts had the lowest TNC. However, the bract nonstructural carbohydrate concentrations almost kept a constant level. Although bracts had lower TNC concentration compared with leaves, bract sucrose represented a higher portion (18%) of TNC of bracts than that of the leaves (10%) (averaged over all sampling dates in the two years). A high fraction of sucrose in bracts is probably beneficial to carbohydrate translocation from bracts to fruits.

Carbon Contribution of Main-Stem Leaf, Sympodial Leaf and Bracts to the Floral Bud

Main-stem and sympodial leaves at fruiting position 1 of MSN 10 had similar abilities for total carbon fixation on a tissue basis, whereas total $^{14}CO_2$ fixation of bracts was only 21% of the leaf (Table 1). However, when considering ^{14}C radioactivities per mg dry tissue (on a DW basis), the main-stem leaf had significantly lower radioactivity ($P \leq 0.05$) among the three $^{14}CO_2$-labeled tissues, with the bracts and subtending sympodial leaf having similar ^{14}C-fixing abilities. The low radioactivity per unit DW for the main-stem leaf was possibly due to shade from upper leaves in the plant canopy (Oosterhuis and Urwiler, 1988). It is possible that some ^{14}C-assimilate produced by the main-stem leaf was exported into other tissues, such as the main stem and adjacent fruiting branches (Brown, 1968).

Although the bracts had the lowest total $^{14}CO_2$ fixation of the three organs labeled, the floral bud of $^{14}CO_2$-labeled bracts had significantly higher ^{14}C radioactivity than those of $^{14}CO_2$-labeled main-stem or sympodial leaves at 6 h after $^{14}CO_2$-feeding treatments. In a 6-h translocation period, the bracts supplied about 18% of the ^{14}C-assimilate fixed to the floral bud, whereas the main-stem leaf and subtending sympodial leaf exported only 1 and 2% of their fixing ^{14}C-assimilate, respectively.

The carbon contributions of these three source tissues to a 20-day-old floral bud at fruiting position 1 were estimated according to the bud radioactivity (Table 1). The bracts subtending a floral bud were the major carbon supplier for the bud, providing about 56% of ^{14}C-assimilate imported into the bud. The main-stem leaf and subtending sympodial leaf provided only 17 and 27% of the ^{14}C-assimilate, respectively, for the floral bud at fruiting position 1.

Although bracts had lower P_n than the leaves during cotton fruit development, Wullschleger and Oosterhuis (1990) pointed out the function of bracts in carbon assimilation may increase under stress conditions, such as water stress and shade. Our study of $^{14}CO_2$ fixation and ^{14}C-assimilate translocation from sources (leaves and bracts) to the sink (floral buds) revealed that the floral bracts were very important for the carbon supply of square development. In the floral bud of a 20-day-old square at fruiting position 1 of MSN 10, about 56% of ^{14}C-assimilate came from subtending bracts, only 17% from the main-stem leaf, and 27% from subtending sympodial leaf (Table 1). Bract assimilate was apparently easier to move into the floral bud compared to the leaf assimilate. This may be related to a shorter pathway between bracts and the floral bud. Additionally, more rapid translocation of assimilate from bracts to the floral bud is also associated with nonstructural carbohydrate composition in bracts. In our studies, we also found that the portion of sucrose and TNC (hexose + sucrose + starch) concentrations in floral bracts was greater than the portion in the leaves (Fig. 4). Therefore, a greater sucrose fraction in the bracts may be one of the causes leading to faster translocation of bract photosynthate into the fruits because sucrose is a major form in which carbon is translocated in plants (Kruger, 1990).

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Table 1. Total $^{14}CO_2$ fixation of main-stem leaf, sympodial leaf and floral bracts, and floral bud radioactivities and percentage of ^{14}C-assimilate translocated into the bud of a square at fruiting position 1 of main-stem node 10 at 6 h after $^{14}CO_2$-labeling in 1997. Each value is mean of 3 replications.

Source tissue	Total $^{14}CO_2$ fixation† (dpm)		Floral bud radioactivity (dpm bud^{-1})	% transported to floral bud ‡
	per tissue	per mg DW		
MSL	753,473 a§	612 b	8,389 b	1.1 b
SSL	700,946 a	1,658 a	13,245 b	1.9 b
Bracts	157,548 b	1,947 a	27,954 a	17.7 a

† Total $^{14}CO_2$ fixation is the sum of radioactivities in the tissues of the petiole and blade of source leaf, and the peduncle, bracts and floral bud of the subtended square for labeled leaves, and the sum of radioactivities in the bud and subtending bracts for labeled bracts.

‡ % transported to floral bud = [floral bud radioactivities (cpm) ÷ total ^{14}C fixation (cpm)] ×100.

§ Means followed by the same letter within a column are not significantly different ($P > 0.05$).

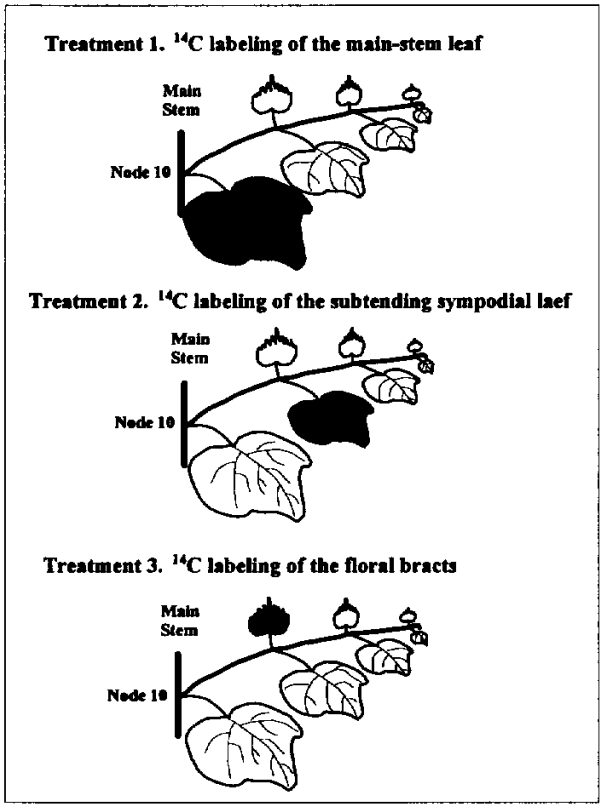

Figure 1. Diagrammatic representation of a cotton sympodial branch from main-stem node 10 and the three treatments of using $^{14}CO_2$ to feed different source tissues in 1997.

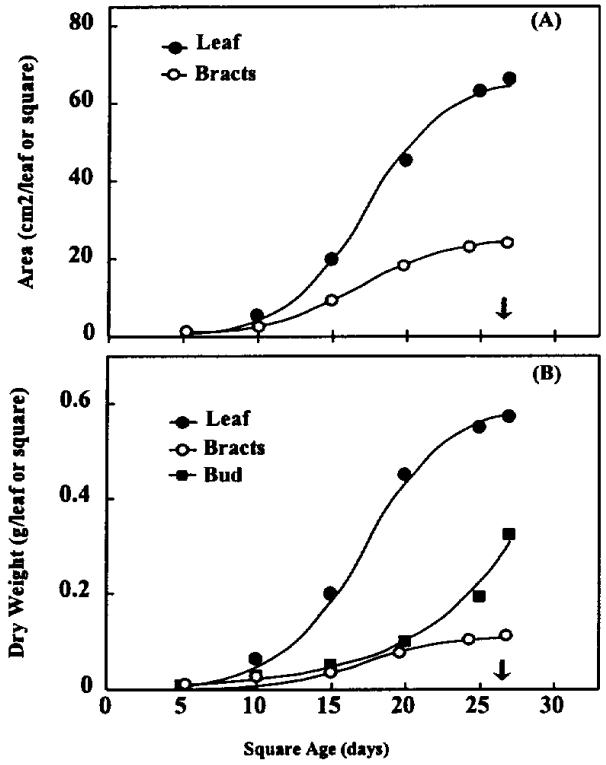

Figure 2. Changes in (A) the areas of the sympodial leaf and floral bracts subtending a square at the fruiting position 1 of MSN 10 and (B) dry weight accumulations of the sympodial leaf, the bracts and the floral bud during the square development. Data are means of 1993 and 1994. Arrows indicate flowering.

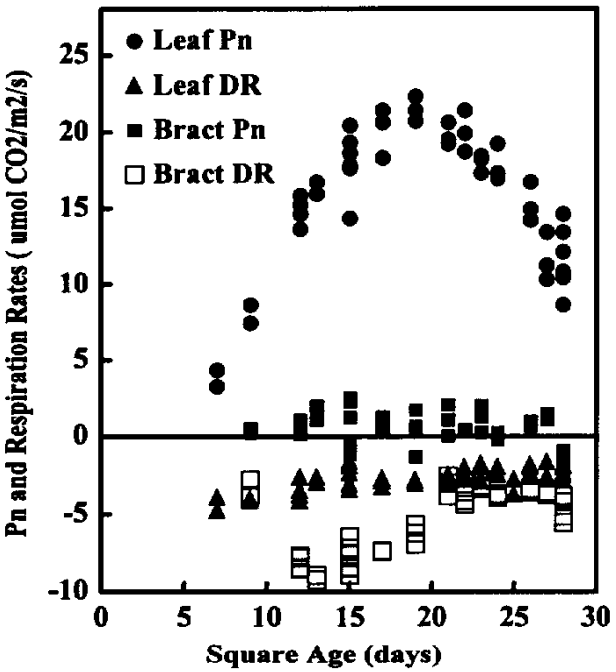

Figure 3. The Photosynthetic (Pn) and dark respiration (DR) rates of floral bracts and sympodial leaves subtending the squares with increasing square ages for greenhouse-grown cotton plants.

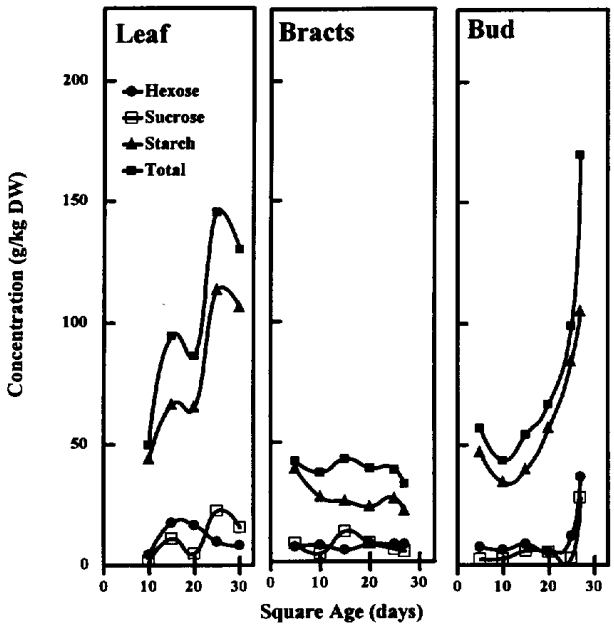

Figure 4. Changes in nonstructural carbohydrate concentrations of subtending leaves, bracts, and floral buds during development of squares at the first fruiting position of main-stem node 10 (means of 1993 and 1994).