Planta (2012) 235:325–336
DOI 10.1007/s00425-011-1511-z

ORIGINAL ARTICLE

Important photosynthetic contribution from the non-foliar green organs in cotton at the late growth stage

Yuan-Yuan Hu · Ya-Li Zhang · Hong-Hai Luo · Wei Li · Riichi Oguchi · Da-Yong Fan · Wah Soon Chow · Wang-Feng Zhang

Received: 20 April 2011 / Accepted: 22 August 2011 / Published online: 9 September 2011

Abstract Non-foliar green organs are recognized as important carbon sources after leaves. However, the contribution of each organ to total yield has not been comprehensively studied in relation to the time-course of changes in surface area and photosynthetic activity of different organs at different growth stages. We studied the contribution of leaves, main stem, bracts and capsule wall in cotton by measuring their time-course of surface area development, O_2 evolution capacity and photosynthetic enzyme activity. Because of the early senescence of leaves, non-foliar organs increased their surface area up to 38.2% of total at late growth stage. Bracts and capsule wall showed less ontogenetic decrease in O_2 evolution capacity per area and photosynthetic enzyme activity than leaves at the late growth stage. The total capacity for O_2 evolution of stalks and bolls (bracts plus capsule wall) was 12.7 and 23.7% (total ca. 36.4%), respectively, as estimated by multiplying their surface area by their O_2 evolution capacity per area. We also kept the bolls (from 15 days after anthesis) or main stem (at the early full bolling stage) in darkness for comparison with non-darkened controls. Darkening the bolls and main stem reduced the boll weight by 24.1 and 9%, respectively, and the seed weight by 35.9 and 16.3%, respectively. We conclude that non-foliar organs significantly contribute to the yield at the late growth stage.

Keywords Different growth stages · O_2 evolution · Photosynthetic enzymes · Surface area · Reproductive biomass

Abbreviations

ATP	Adenosine triphosphate
Chl	Chlorophyll
DAA	Days after anthesis
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetate
MDH	Malate dehydrogenase
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
PEPC	Phosphoenolpyruvate carboxylase
RuBPC	Ribulose 1,5-bisphosphate carboxylase

Y.-Y. Hu · Y.-L. Zhang · H.-H. Luo · W. Li · W.-F. Zhang (✉)
The Key Laboratory of Oasis Eco-agriculture, Xinjiang Production and Construction Group, Shihezi University, Shihezi 832003, People's Republic of China
e-mail: zhwf_agr@shzu.edu.cn; zwf_shzu@163.cm

R. Oguchi · W. S. Chow
Division of Plant Science, Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, Canberra, ACT 0200, Australia

D.-Y. Fan
State Key Laboratory of Vegetation and Environment, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, People's of China

Introduction

More than 90% of crop biomass is derived from photosynthetic products. Consideration and modeling of photosynthesis are usually restricted to leaves, but many parts of the plant besides leaves contain chlorophyll and, therefore, capture light energy to perform photosynthesis. Evidence of photosynthetic activity of non-foliar organs has been found in, for example, the panicles of rice

(Ishihara et al. 1991), the ear of wheat (Singal et al. 1986; Araus et al. 1993; Li et al. 2006), the spikes of barley (Duffus and Cochrane 1993), and the fruit in tomato (Xu et al. 1997). It is now accepted that photosynthesis of non-foliar green organs is an important, additional contribution to carbon acquisition and yield (Aschan and Pfanz 2003).

At its late growing stage, cotton bears large dark-green bolls, which in their early development are covered by bracts. Both the bracts and the capsule wall of bolls have photosynthetic function and contribute to carbon gain (Constable and Rawson 1980; Wullschleger and Oosterhuis 1990, 1991; Wullschleger et al. 1991). Zhang et al. (2010) and Du et al. (2009) also showed that main stems and capsule wall have photosynthetic activity and could contribute to canopy photosynthesis and yield of cotton. It is empirically known that cotton leaves tend to senesce earlier than other organs at the late growth stage; therefore, the contribution of non-foliar organs to yield can be important, especially at the late growth stage.

However, there has been no direct measurement of the relative contribution of non-foliar green organs to yield at the different growth stages in cotton, particularly in relation to the surface area and enzyme activity of the organs that support photosynthesis. Although the photosynthetic capacity of non-foliar organs of cotton has been analyzed by the gas exchange technique or in an anatomical investigation (Wullschleger and Oosterhuis 1990; Wullschleger et al. 1991; Bondada et al. 1994; Bondada and Oosterhuis 2000), there is little information about the activities of carbon fixation enzymes in cotton, as has been studied in the ear of wheat (Singal et al. 1986; Li et al. 2006).

Our aims were to investigate how the activity of photosynthetic enzymes differ between the leaf and non-foliar green organs and how the non-foliar organs of cotton contribute to photosynthesis of the whole plant, considering the time-course of changes in surface area and enzyme activity of each organ at different growth stages. We determined the surface area and dry matter of the leaves and non-foliar organs at the peak flowering stage (85 days from sowing), the intermediate stage (98 days from sowing) and the full bolling stage (120 days from sowing). We also compared the photosynthetic rate and the activity of carboxylation enzymes (RuBPC and PEPC) of leaves, bracts, main stem and capsule wall on different days after anthesis. Using the oxygen evolution rate per area and surface area of each organ at these time points, we calculated the contribution of each organ to whole plant photosynthesis. In addition, the effect of darkening non-foliar organs was studied in an attempt to reveal the relative contribution to the yield of the whole plant.

Materials and methods

Plant materials and growth conditions

Our study was conducted at an experimental field of Shihezi Agricultural College, Shihezi University, Xinjiang, China (45°19′N, 86°03′E) in 2009 and 2010. Cotton (*Gossypium hirsutum* L. cv. Xinluzao 13; seeds obtained from Agriculture Science Research Institute, Kuitun City, Xinjiang Province, China) was grown under field conditions. Seeds were sown on 22 April in 2009 and 24 April in 2010 in rows 12 cm apart at a plant density of 1.8×10^5 ha^{-1}. The plot was drip irrigated and maintained well-watered throughout the whole season. Pest and weed control was carried out according to the local standard practice. The experimental design was completely randomized with three replications. Materials for the measurements of O_2 evolution, photosynthetic enzymes and chlorophyll content were collected from the penultimate main leaves after tip pruning (July 5, 2009 and July 10, 2010), as well as from corresponding bolls, their associated bracts, and main stems under the main-stem leaves. To minimize age-related variability in the measured parameters, approximately 400 first position fruits at penultimate main stem leaves were labelled with plastic tags at anthesis (began July 25, 2009 and July 10, 2010). Ambient photosynthetically active irradiance was measured with an external quantum sensor attached to an infrared gas exchange analyzer (Li-6400; Li-Cor Inc., Lincoln, NE, USA) by positioning the sensor horizontal to the ground on a sunny day in July.

Surface area and biomass

The surface area of leaves, subtending leaves and bracts was measured using a leaf area meter (LI-300, Li-Cor, Lincoln, NE, USA). Vernier calipers were used so that the surface area of stalks (including carpopodium and petiole) could be calculated from the length and diameter of the stem section between the upper and lower leaves. At the different growth stages, the surface area of the bolls was traced on white paper with the sectioned capsule wall, disregarding ones which were younger than 15 days after anthesis (DAA); then the capsule wall surface area was calculated by dividing the paper weight of the traced area by the paper weight per unit area. After drying to constant dry weight at 85°C ($\geq$24 h) dry weights were determined. These measurements were repeated at the peak full flowering (85 days from sowing), the intermediate stage (98 days from sowing) and the full bolling stage (120 days from sowing). We did not include the surface area or biomass of the fallen leaves or other organs.

Nitrogen content of all green organs

For the measurement of the nitrogen content (Table 1), leaves, bracts and capsule wall were harvested on the same day, about 20 DAA of the associated boll. Total nitrogen content of the dried tissues was determined according to the micro-Kjeldahl method (Schuman et al. 1972).

Measurements of photosynthetic O_2 evolution

The leaves, bracts, bolls and the main stem under the main leaf were removed from the plant at 09:30 a.m., placed on moistened cloth, and then taken to the lab as quickly as possible. Samples of leaves, bracts, main stem and capsule wall were cut into small sections. Oxygen evolution capacity of the sectioned samples was measured at 25°C using a ChloroLab2 liquid-phase O_2 electrode system (Hansatech Instruments, Norfolk, UK), in which the CO_2 was saturating for photosynthesis. The reaction mixture (2.0 mL) was composed of 20 mM $NaHCO_3$ and 60 mM Tris–HCl (pH 7.5). Illumination (1,200 μmol photons m^{-2} s^{-1}) was provided by red light-emitting diodes. The area and fresh weight of leaves, bracts, main stem and capsule wall were also recorded at each measurement time point. All the O_2 evolution capacities were calculated on an area basis (μmol O_2 m^{-2} s^{-1}) and fresh weight basis (μmol O_2 g FW^{-1} min^{-1}). Data were averaged from six replicates.

Extraction and assay of key enzymes in the Calvin cycle and soluble proteins

The key photosynthetic enzymes of leaves, bolls, bracts and main stems were investigated on 5, 20 and 50 DAA. The extraction of enzymes was carried out according to Sayre et al. (1979) with slight modifications. Green tissue (0.2 g) was ground with a mortar and pestle (4°C) containing a small amount of sand and 1.0 mL of grinding media consisting of 0.1 M Tris–HCl (pH 7.8), 100 mM $MgCl_2$, 1 mM EDTA, 20 mM mercaptoethanol, 100 kg m^{-3} glycerin, and 10 kg m^{-3} polyvinylpyrrolidone. Following centrifugation at 15,000*g* for 10 min at 4°C, the supernatant was used for enzyme assays. RuBPC activity was assayed by the method of Camp et al. (1982). An enzyme extract was added to a reaction mixture that contained 50 mM Tricine–NaOH (pH 7.9), 10 mM KCl, 1 mM EDTA, 2 mM dithiothreitol (DTT), 0.2 mM NADH, 5 mM ATP, 15 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM phosphocreatine, 2 units per mL creatine phosphokinase, 4 units per mL each of NAD-dependent glyceraldehyde-3-P-dehydrogenase and 3-P-glycerate kinase in a final volume of 1 mL. The mixture was incubated at 25°C for 5 min. Reactions were initiated by the addition of 0.5 mM RuBP.

For PEPC activity assay, about 0.2 g of each sample was frozen in liquid nitrogen and pulverized with a mortar and 1.0 mL of grinding medium consisting of 1 mM Tris–H_2SO_4 (pH 8.2), 7 mM mercaptoethanol, 1 mM EDTA, 5% (*v*/*v*) glycerol, and 1% (*w*/*v*) insoluble polyvinylpyrrolidone. The homogenates were filtered through cheesecloth, and the filtrates were centrifuged at 20,000*g* for 15 min at 4°C. The supernatants were used for assays of enzymatic activity. PEPC activity was measured spectrophotometrically at 340 nm using a spectrophotometer (U-3900, Hitachi, Tokyo, Japan) and by coupling the PEP reaction to the oxidation of NADH with malate dehydrogenase (MDH) according to Blanke and Ebert (1992). The enzyme extract was added to a solution which, in a 1.5 mL final volume contained 50 mM Tris–HCl (pH 7.8), 10 mM $MgCl_2$, 0.25 mM EDTA, 5.0 mM $NaHCO_3$, 2.0 mM DTT, 4 unit MDH, 0.1 mM NADH, and 2.0 mM PEP. The reaction was started by the addition of tissue extract. For consistency with the oxygen evolution rate, each enzyme activity was calculated on both an area basis and fresh weight basis.

The soluble protein content of leaves was measured according to the Coomassie brilliant blue G_{250} method described by Read and Northcote (1981). The soluble protein content was measured by a spectrophotometer at 595 nm.

Chlorophyll content of all green organs

The content of chlorophyll of plant organs was determined in discs from leaves, bracts or capsule wall tissue removed by a calibrated metallic borer (4 mm diameter), or a main stem segment of known size. Discs of the green organs were extracted in 80% (*v*/*v*) acetone for 24 h at room temperature in the dark. The absorbance of an extract was measured with a spectrophotometer, and the chlorophyll content was calculated according to Lichtenthaler (1987).

The total capacity for O_2 evolution of the green organs to the whole plant

The contribution of photosynthesis in each green organ to the whole plant was calculated as the product of the surface

Table 1 The nitrogen content of main leaves and their corresponding bracts and capsule wall (on 20 days after anthesis) expressed on the basis of surface area or dry weight (%)

Organ	Nitrogen content (g m^{-2})	Nitrogen content (%)
Leaves	2.96 ± 0.02[b]	3.52 ± 0.02[a]
Bracts	0.58 ± 0.02[c]	2.34 ± 0.01[b]
Capsule wall	12.38 ± 0.13[a]	2.08 ± 0.02[c]

The values are means ± SE of three replicates. Between organs, bars with the same letter beside them are not significantly different ($P \leq 0.05$)

area and the O_2 evolution rate per area (Figs. 1a, c, 2a, b, respectively), at the peak flowering stage and the late full bolling stage (Table 2). Since the surface area of the capsule wall at 5 DAA was too small for measurement of photosynthesis rate, for the calculation of the peak flowering stage (July 17) the O_2 evolution rate of the capsule wall was chosen as that at 15 DAA, while the average value of 5 DAA and 15 DAA was taken as the average photosynthetic rate of the other organs. We used the instantaneous area at the peak flowering stage, shown in Fig. 1a, for the surface area. For the calculation of the late growth stage (August 22), when the bolls in different parts of the plant were at different growth stages, the average value from 20 DAA to 50 DAA was taken as the average photosynthetic capacity of each organ. The instantaneous area at the full bolling stage, which was shown in Fig. 1c, was used as the surface area. The percent contribution of photosynthesis in each green organ to the total plant was estimated by the total photosynthesis rate of each organ divided the photosynthesis rate of the whole plant (the sum of all organs).

The relative contribution to the capsule wall weight, seed weight, and fibre yield

From 15 days after emergence of each boll to harvest, the bolls (capsule wall plus bracts) of nine plants were kept in the dark by covering with aluminium foil, following the method of Araus et al. (1993). Similarly, the main stem of nine other randomly selected plants were kept in the dark from 1 August, about a fortnight after peak flowering. Nine other non-darkened plants were tagged at this point. The dry capsule wall weight per boll, seed weight per boll and fibre weight per plant were measured for the control, boll-darkened, and main stem-darkened plants (on 27 September). Although darkening by covering the organ with aluminium may not only affect the light intensity but also the temperature and composition of the air around the organ, it is a common method to assess the relative contribution of the green organs photosynthesis to the plant. In our study, we attempted to limit the build-up of ethylene and water vapour around the non-foliar organs by having small holes (at least 15 mm apart, and representing about 0.3% of the covered area) in the aluminium foil.

$$\text{Relative contribution } (\%) = 100 \times (\text{control yield} - \text{darkened yield})/\text{control yield}$$

Data analysis

All data were subjected to analysis of variance (ANOVA) using DPS statistical software (v.7.55, Refine Information Tech. Co. Ltd. Hangzhou, Zhejiang, China). The data are presented as the mean ± standard error. The significance of differences between mean values was determined with Tukey's test. Differences at $P \leq 0.05$ were considered significant.

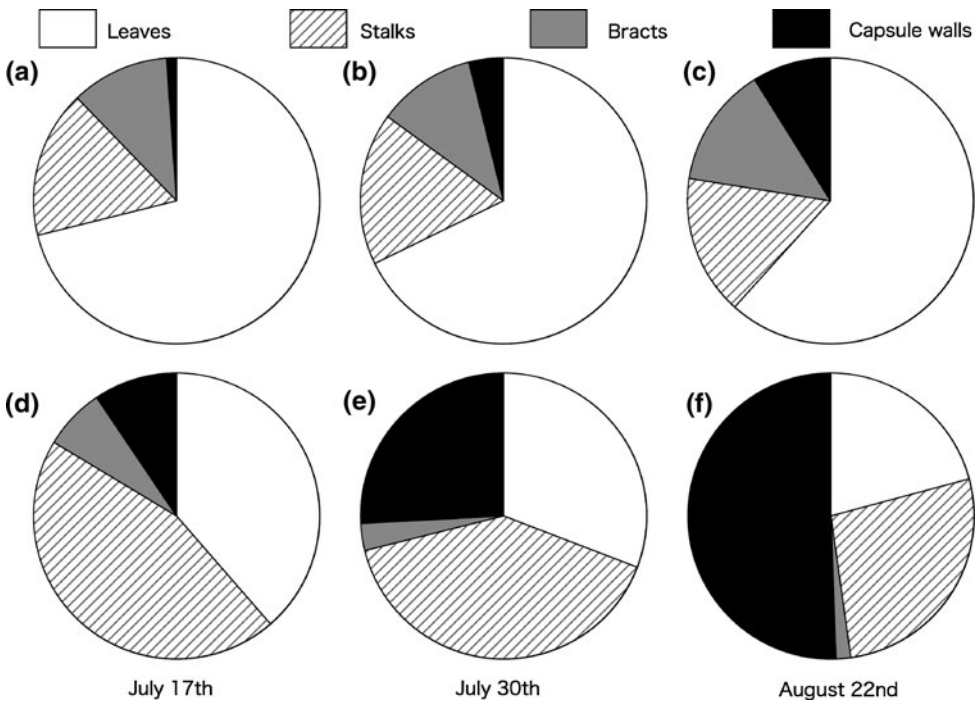

Fig. 1 Relative contributions of leaves, bracts, stalks, and bolls to the total surface area of a single cotton plant (**a**–**c**) and to the plant dry weight (**d**–**f**) at three different growth stages: peak flowering stage (**a**, **d**), an intermediate stage (**b**, **e**) and full bolling stage (**c**, **f**)

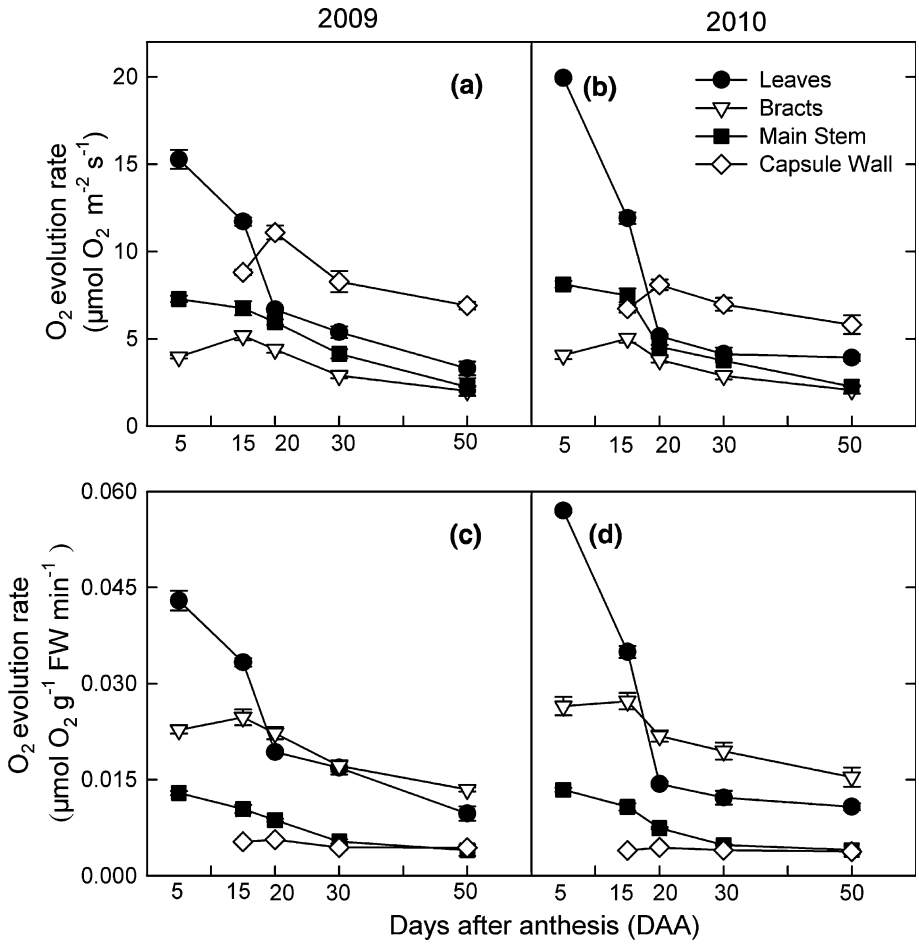

Fig. 2 CO_2-saturated oxygen evolution rates of leaves, bracts, main stem and capsule wall expressed on both surface area (**a**, **b**) and on fresh weight (**c**, **d**) bases at various days after anthesis in 2009 and 2010. Measurements were made at an irradiance of 1,200 μmol photons m^{-2} s^{-1}

Table 2 Photosynthesis rates of whole leaves, whole bracts, whole stalks and whole capsule walls (μmol O_2 s^{-1}) at the peak flowering stage and at the late full bolling stage in 2010

Organ	Photosynthesis rate (μmol O_2 s^{-1}) at peak flowering stage	Photosynthesis rate (μmol O_2 s^{-1}) at late full bolling stage
Leaves	4.78 (85.9%)	1.24 (63.6%)
Stalks	0.542 (9.7%)	0.247 (12.7%)
Bracts	0.222 (4.0%)	0.183 (9.4%)
Capsule wall	0.020 (0.4%)	0.279 (14.3%)

The percent contribution of each green organ to photosynthesis rate of whole plant is shown in parentheses. We assumed that the organs were exposed to the saturating irradiance 1,200 μmol photon m^{-2} s^{-1} in the field

Results

Surface area and biomass of green organs

As a percentage of total plant surface area at the peak flowering stage, all the non-foliar green organs together amounted to 28.8%, while the area of leaves dominated at 71.2% (Fig. 1a). This proportion changed during growth; at the full bolling stage the non-foliar organs increased to 36.2% (Fig. 1c). The most dramatic increase was the surface area of the fruits which increased by 97.7% at the late full bolling stage compared with that at the peak flowering stage. At the peak flowering stage, the non-foliar organs accounted for 61.3% of the total dry matter; the fraction increased to 79.1% at the full bolling stage, the bolls (capsule wall, fibre and seeds) amounting to about 50% (Fig. 1f).

O_2 evolution capacity

On an area basis, the rate of oxygen evolution in main leaves showed a pronounced decrease during the development of bolls (Fig. 2a, c). By comparison, bracts reached an obvious peak in photosynthesis (5.0–5.2 μmol O_2 m^{-2} s^{-1}) at about 15 d after anthesis, which was about 42–44% of that of the main leaves at 15 DAA (Fig. 2a). Beyond this time, bracts followed a declining trend with age. The main stem maintained higher O_2 evolution rates than bracts during ontogeny, but the O_2 evolution rate decreased monotonically with age. The O_2 evolution rate of capsule wall on an area basis was

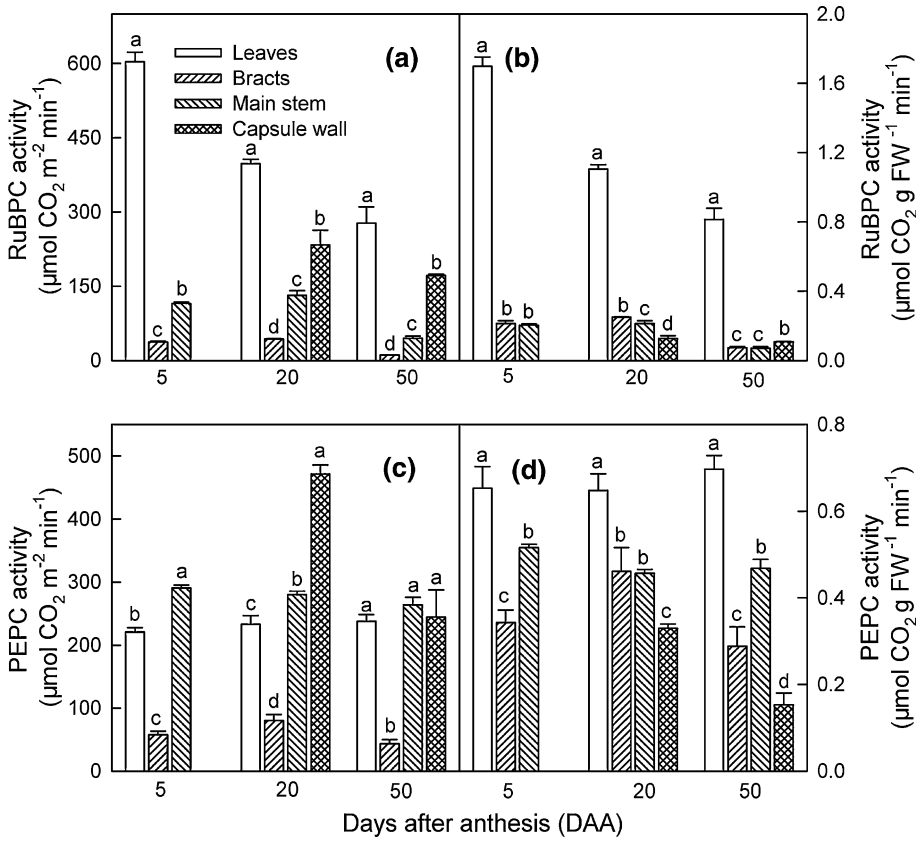

Fig. 3 Activities of Ribulose-1,5-biphosphate carboxylase (RuBPC) expressed on the basis of area (**a**) or fresh weight (**b**) in leaf and non-foliar green organs of cotton at various days after anthesis in 2010. Activities of phosphoenolpyruvate carboxylase (PEPC) expressed on the basis of area (**c**) or fresh weight (**d**) in leaf and non-foliar green organs of cotton at various days after anthesis in 2010. Between organs, *bars with different letters* had statistically significant difference ($P \leq 0.05$)

higher than that of leaves during the late stage of boll development, having peaked at 20 DAA.

On a fresh weight basis, the photosynthesis capacity of both leaves and the main stem decreased with age. Bracts reached an obvious peak in photosynthesis at about 15 DAA, and then followed a declining trend with age. However, the photosynthesis rate of the capsule wall decreased only slightly compared with that of leaves in 2009, and was stable in 2010 (Fig. 2b, d).

Photosynthetic enzymes and soluble protein content at different growth stages

Both on an area basis and on a fresh weight basis, the RuBPC activity in leaves showed a significant decreasing trend during the development of bolls. The RuBPC activity in non-leaf organs rose at the beginning; thereafter, the value sharply decreased from 20 DAA (Fig. 3a, b). On an area basis, the decrease in RuBPC activity from 20 DAA to 50 DAA was 30.1% in leaves, 74.3% in bracts, 65.7% in the main stem and 26.5% in the capsule wall (Fig. 3a). On a fresh weight basis, the decrease in RuBPC activity from 20 DAA to 50 DAA was 26.3% in leaves, 70.2% in bracts, 66.7% in the main stem and 4.9% in the capsule wall (Fig. 3b).

Compared to leaves, the PEPC activity at 20 DAA in the main stem and capsule wall on an area basis were approximately 1.2- and 2-fold higher, respectively (Fig. 3c). However, the PEPC activity on a fresh weight basis in leaves was significantly greater than in non-foliar organs during the growth stages (Fig. 3d). The trend of soluble protein in the various green organs was mostly the same as the RuBPC activity during the growth stages (Fig. 4). On an area basis, soluble protein was higher in the capsule wall than that in leaves during the latter growth stages (Fig. 4a). On a fresh weight basis, the decrease in soluble protein from 20 to 50 DAA was 31.3% in leaves, 75% in bracts, 22.2% in main stem and 24.6% in the capsule wall (Fig. 4b).

Nitrogen content of leaves, bracts and capsule wall

In the three organs, on an area basis, the nitrogen content was highest in the capsule wall, and lowest in the bracts (Table 1), consistent with the soluble protein content (Fig. 4a). On a dry weight basis, the nitrogen content was highest in leaves, consistent with the soluble protein content on a fresh weight basis (Fig. 4b), and lowest in the capsule wall.

Chlorophyll analyses

Both the Chl content, based on the area or fresh weight analyses, of non-foliar organs (bracts, main stem and

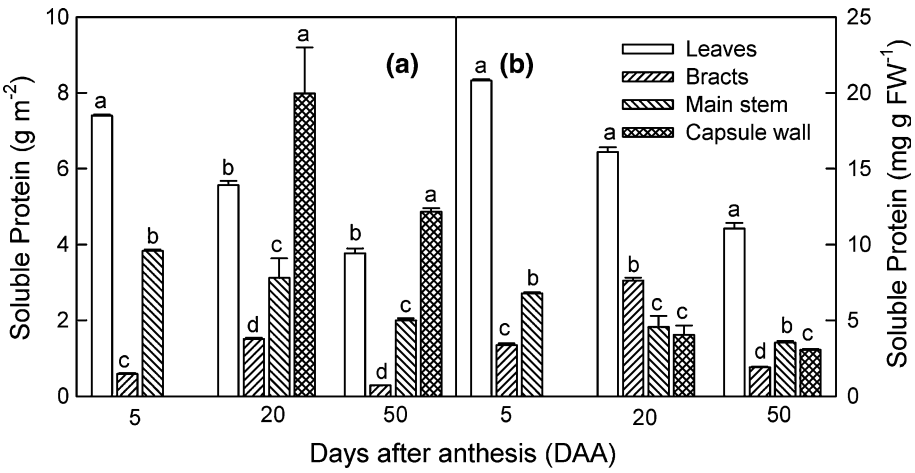

Fig. 4 Soluble protein content expressed on the basis of area (**a**) or fresh weight (**b**) in the leaf and non-foliar organs of cotton at various days after anthesis in 2010. Between organs, *bars with the same letter* above them are not significantly different ($P \leq 0.05$)

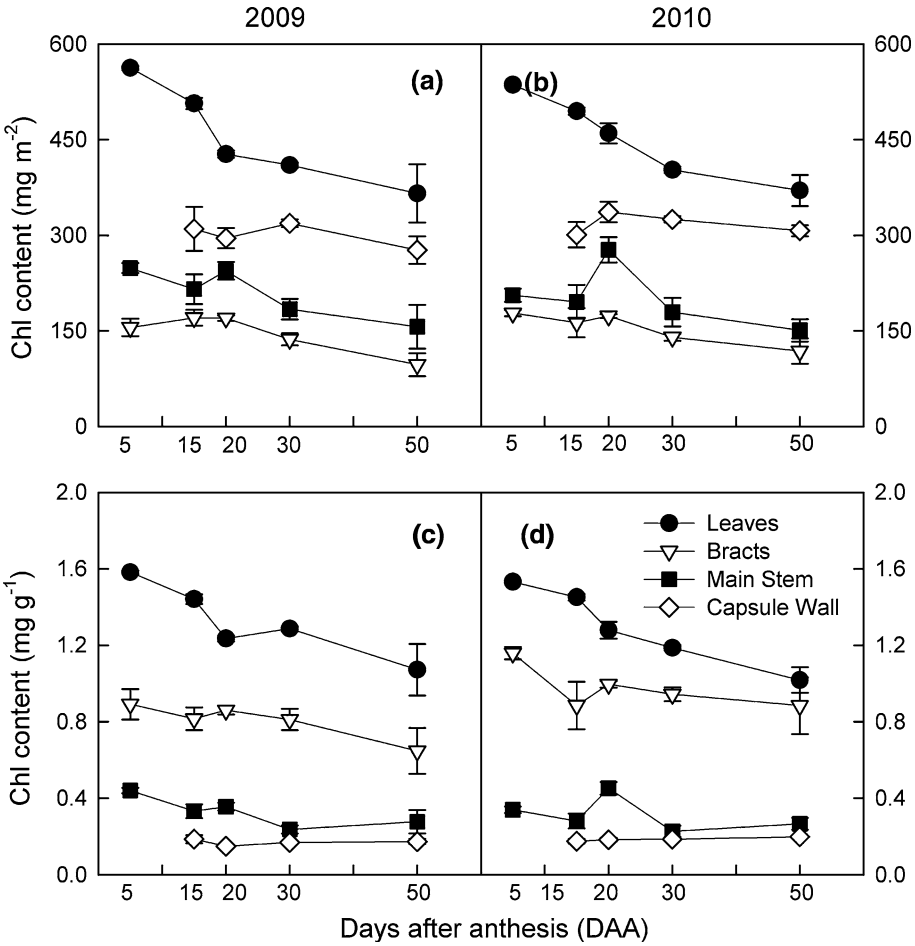

Fig. 5 Changes in total chlorophyll (Chl) content of leaves, bracts, main stem and capsule wall expressed on the basis of surface area (*upper panels*) or fresh weight (*lower panels*) during various times after anthesis in 2009 (**a**, **c**) and 2010 (**b**, **d**)

capsule wall) were significantly lower than those of leaves (Fig. 5a, b). On a surface area basis, the Chl content in leaves decreased with age. However, the Chl content in non-foliar organs slightly increased in the beginning, followed by a small decrease with age (Fig. 5a). On a fresh weight basis, the decline in the Chl content was less in non-foliar organs than in leaves (Fig. 5c, d).

The relative photosynthetic contribution of the different organs to the total plant

The oxygen evolution rate can be an indication of photosynthetic activity (Caley et al.1990). Based on the mean surface area and the O_2 evolution rate of each Chl-containing organ during the growth stages (Figs. 1, 2), the

Table 3 Effect of darkening the boll (capsule wall and bracts) from 15 days after emergence to maturity and the main stem from 1 August to 15 September on boll number, boll dry weight, seed dry weight, lint dry weight and dry weight of capsule wall per plant, on 15 September

Treatment	Boll number per plant	Boll weight per plant (g)	Seed weight per plant (g)	Lint weight per plant (g)	Capsule weight per plant (g)
Control	10.4 ± 1.8a	64.3 ± 11.7a	30.6 ± 8.8a	17.1 ± 2.2a	16.7 ± 3.8a
Boll (capsule wall plus bracts) darkened	8.1 ± 1.7b (−22.1%)	48.8 ± 11.8b (−24.1%)	19.6 ± 7.5b (−35.9%)	15.6 ± 0.9a (−8.8%)	12.5 ± 3.6b (−25.1%)
Main stem darkened	10.8 ± 2.2a (3.8%)	58.5 ± 8.6ab (−9.0%)	25.6 ± 7.5ab (−16.3%)	17.0 ± 2.2a (−0.5%)	15.8 ± 3.7ab (−5.4%)

The values are means ± SE of nine replicates. Values in parentheses are the percentage decrease due to placing the organs in darkness. Between organs, bars with the same letter beside them are not significantly different ($P \leq 0.05$)

relative photosynthetic contribution of the different parts to the total plant in terms of carbon assimilation was estimated by multiplying the O_2 evolution rate per area by the total area of the respective organ (Table 2), following the method of Aschan et al. (2005) who used the CO_2 exchange rate. As shown in Table 2, the photosynthetic contribution of stalks and the bolls (bracts plus capsule wall) were 9.7 and 4.4% of the total, respectively, at the peak flowering stage. At the late full bolling stage, the total photosynthetic contribution of the stalks and bolls were 12.7 and 23.7%, respectively (Table 2). Overall, the non-foliar green organs contributed about 14 and 36.4% of photosynthesis to the whole plant at the peak flowering stage and full bolling stage, respectively.

Relative contribution of the green organs to the yield from the darkening experiment

The relative photosynthetic contribution of bolls (capsule wall plus bracts) and the main stem to boll dry weight, seed dry weight, lint dry weight and capsule dry weight per plant was assessed by the reduction of their weight by darkening bolls or the main stem (Table 3). The plants with darkening of the bolls had significantly reduced boll number per plant compared to non-darkened plants. Boll weight was reduced by the darkening of bolls and the main stem, by 24.1 and 9%, respectively. Seed weight per plant was reduced by the darkening of bolls and the main stem, by 35.9 and 16.3%, respectively. The contribution of bolls to fibre was about 8.8%. The bolls contributed 25.1% of the dry weight in the capsule wall, while the main stem contributed 5.4%.

Discussion

Differences in O_2 evolution rate and carboxylation enzymes of different green organs in cotton

The photosynthetic performance of the non-foliar organs is more and more studied by the chlorophyll (Chl) fluorescence techniques (Aschan and Pfanz 2003, 2006; Aschan et al. 2005). However, chlorophyll fluorescence data do not necessarily represent the photosynthetic status of the whole leaf tissue, especially in thick leaves, a deficiency which has been shown by close comparisons of the electron transport rates measured with chlorophyll fluorescence and with gas-exchange (Kingston-Smith et al. 1997; Tsuyama et al. 2003). The measuring light intensity diminishes with increasing depth in the tissue, and the possibility of re-absorption of the chlorophyll fluorescence signal is increased for the chlorophyll fluorescence emitted in the deeper tissue (Evans et al. 1993; Evans 2009). Oguchi et al. (2011) measured photoinhibited leaves with various conventional fluorometers, and concluded that fluorometers with red-measuring light probe deeper into leaf tissue than those with blue-measuring light. Bondada et al. (1994) have found that the capsule wall (1,013 μm) was much thicker compared with the leaves (152 μm) in cotton (*G. hirstum* L.). Accordingly, although PSII quantum yield in the capsule wall was estimated as 33–64% of that in leaves with the chlorophyll fluorescence techniques (Zhang et al. 2010), their conclusion may be subjected to some uncertainties. Hence, we aimed to measure the photosynthetic capacity of the whole tissue by using a liquid-phase oxygen electrode.

The capacity for oxygen evolution on an area basis in our study was substantially higher in the capsule wall than that in leaves at 20 DAA (Fig. 2). At about 20 DAA, the N content per area was also significantly (about 4.2-fold) higher in the capsule wall than in leaves (Table 1). Our findings are consistent with those of Wullschleger and Oosterhuis (1990). More than half of the leaf nitrogen is allocated to photosynthetic proteins (Evans 1989; Makino and Osmond 1991), such as Calvin-Benson cycle enzymes, coupling factor and electron carriers; these proteins are important in determining the photosynthetic capacity, which generally strongly correlates with the total amount of leaf nitrogen per unit leaf area (Field and Mooney 1986; Evans 1989; Walcroft et al. 1997). Because nitrogen content on a dry weight basis (%) of the capsule wall was significantly lower than that of leaves, the higher nitrogen

content per area was due to the much greater thickness of capsule wall than leaves. Thus, we tentatively attribute the higher oxygen evolution rate of capsule wall per area to its greater thickness.

On an area basis, the capsule wall had significantly higher soluble protein content than leaves at the late growth stage (Fig. 4a); however, the RuBPC activity of the capsule wall was significantly lower than that of leaves (Fig. 3a). RuBPC is usually the major soluble protein of photosynthetic tissue, comprising about 50% (Makino et al. 1983). Possibly, the capsule wall had an abundance of soluble proteins (including PEPC) other than RuBPC. Indeed, at 20 DAA, PEPC activity per area in the capsule wall was 200% of that in leaves (Fig. 3c). At the same stage, the RuBPC activity per area in the capsule wall was 59% of that in leaves. We cannot directly equate differential activities of the two enzymes with their differential protein contents. However, if for simplicity, one assumes that equal enzyme activity means equal protein content, then the combined protein content of RuBPC and PEPC in the capsule as a percentage of that in the leaves should be between 59 and 200%. Indeed, the soluble protein content in the capsule wall was about 141% of that in the leaves. Similarly, the soluble protein content in each non-foliar organ as a percentage of that in the leaves generally lies between the percentage in terms of RuBPC activity and the percentage in terms of PEPC activity (Fig. 3), at each growth stage, and on an area basis or fresh weight basis.

There is another possible reason for the RuBPC per area being lower in the capsule wall compared with the leaves, while the soluble protein content per area was higher in the capsule wall. Some of the RuBPC, together with other soluble proteins, might merely serve to store nitrogen in the form of protein, without being fully active (Warren and Adams 2004). Even in leaves, proteins are probably better nitrogen stores than amino acids because the latter may increase the attractiveness of foliage for insect herbivores (Prestidge and McNeill 1983; Cockfield 1988). Warren and Adams (2002) suggested that the over-investment in Rubisco by *Pinus pinaster* is an adaptation to temporal variation in N supply which may help growth and photosynthesis during periods of N deficiency. Similarly, we suggest that the abundant soluble protein in capsule wall provides a more metabolically compatible means of N storage that is less vulnerable to insect attack than leaves, thereby serving as a useful nutrient supply when needed. To test this hypothesis, further studies are necessary to explore whether the capsule wall is less susceptible in nitrogen deficiency.

High PEPC activity as well as RuBPC activity was reported in soybean pods (on a fresh weight basis, Hedley et al. 1975) and tomato fruits (on a protein basis, Bravd et al. 1977). PEPC was more active on a Chl basis in ear parts than in the flag leaves (Singal et al. 1986). Our results showed that the PEPC activity in non-foliar organs was significantly lower than in leaves on a fresh weight basis (Fig. 3d), although the PEPC activity in main stem and capsule wall was higher than in leaves on an area basis (Fig. 3c). When expressed on a Chl basis, our data of 2010 (Figs. 3d, 5d) show that PEPC activity was higher in the capsule wall and main stem than in leaves, consistent with the findings of Singal et al. (1986).

Photosynthetic parameters of different green organs of cotton at the different growth stages

In this study, the surface area of non-foliar organs increased from 28.4% of the total at the peak flowering stage to 38.2% at the full bolling stage (Fig. 1a). The surface area of green non-foliar organs is an important factor which affects the dry matter production. For example, the sepals of *Helleborus viridis* L. are a major source of assimilates, amounting to 56% of the total plant surface area in the early spring, while the basal leaves emerged late during fruit development (Aschan et al. 2005). Although the floral organs constitute a relatively small fraction of the entire biomass, the green patterned inner tepals in snowdrop (*Galanthus nivalis* L.) contribute significant photosynthetic activity, thereby providing the flower and the developing seeds with photo-assimilates (Aschan and Pfanz 2006). Thus, because of the early senescence of leaves in cotton, we suggest that the dramatically increased surface area of the capsule wall and, to some extent, the bracts (Fig. 1) might contribute substantially to total plant dry weight at the full bolling stage.

According to a previous study, maximum net photosynthesis rate in cotton leaves is achieved about 15 days after unfolding, but photosynthesis is maintained at this rate for only 12 days, after which the rate of net photosynthesis declines, reaching near zero because of leaf senescence by about 70 days (Constable and Rawson 1980). A short time before flowering, subtending leaves start to emerge. According to Wullschleger and Oosterhuis (1990), the subtending leaf at the time of anthesis is 18 days old at the eighth node on the main stem. Similarly, we also usually found that the subtending leaf was about 20 days old at the time of anthesis. Oosterhuis et al. (1983) suggested that the nitrogen content of main stem leaves and subtending leaves begin to decrease at about 30 days and 70 days from sowing, respectively. On the other hand, the photosynthetic rate of the capsule wall and bracts was largely maintained after flowering (Fig. 2). When main leaves begin to senesce after the bolls emerge, the major photosynthetic role needs to be assumed by the young subtending leaves, and other non-foliar organs. Thus, we suggest that the dramatically increased surface area of the bracts and the capsule wall, both of which maintained their

photosynthesis rates for a longer period than leaves, would compensate for the loss of leaf photosynthetic activity at the late full bolling stage.

In the present study, the RuBPC activity of non-foliar organs was largely maintained after anthesis, even when the RuBPC activity of leaves was decreasing, especially from 5 to 20 DAA (Fig. 3). We suggest that photosynthesis of the non-foliar organs may play an important role in the development of the bolls, especially at the late growth stage. Although the photosynthetic rate of leaves and main stem were decreasing at the late growth stage (Fig. 2), their PEPC activity was maintained. This may suggest that PEPC was not acting to maintain photosynthetic activity, but that it had an as-yet unidentified function.

Chlorophyll plays an important role in the light absorption and energy transduction, which is the basis of photosynthesis. In our study, the decrease in Chl content of non-foliar organs was generally less than that of leaves (Fig. 5). Degradation of photosynthetic pigments is a typical characteristic of leaf senescence. The smaller decrease in O_2 evolution rate and Chl content in non-foliar organs indicates slower senescence than in leaves at the late growth stage.

Contribution of photosynthesis in non-foliar green organs to yield

There are several physiological studies of photosynthesis in non-foliar organs in cotton (Wullschleger and Oosterhuis 1990; Bondada et al. 1994; Bondada and Oosterhuis 2000); however, the contribution of non-foliar organs to yield is still not well quantified. In blueberry fruit (*Vaccinium ashei* Reade), Birkhold et al. (1992) estimated that photosynthesis contributes about 15% of the total carbohydrate required for fruit growth. In rice, 20–30% of the carbohydrates accumulated in the grains are derived from CO_2 assimilated in the panicles after their emergence (Ishihara et al. 1991). The total contribution of non-foliar green organs, including ears and peduncles, accounts for about 40–50% of grain mass per ear (Araus et al. 1993; Wang et al. 2001). Indeed, the physiological importance of photosynthesis of green stems and other green parts in cotton cannot be neglected, especially at the late stage of growth.

When leaves were decreasing their photosynthetic capacity and percentage of surface area, non-foliar organs mostly maintained their photosynthetic rate and dramatically increased their surface area. Stalks had constant surface area (15.6–17.3% of the total surface area) during the growth stages (Fig. 1a–c). Assuming the middle value of the photosynthetic capacity from 5 to 15 DAA as the average photosynthetic rate of the main stem at the peak flowering stage, we found that the stalks contributed 9.7% of the total O_2 evolution capacity of the whole plant at the peak flowering stage, and 12.7% at the late full bolling stage (Table 2). Similarly, Du et al. (2009) reported that the stems in two high-yielding hybrid cotton cultivars contributed about 9–11% of the total canopy photosynthesis at the late growth stage. From the flowering stage to the full bolling stage, the surface area of the bracts and capsule wall strongly increased (Fig. 1a–c). If we take the average photosynthetic capacity from 20 to 50 DAA (Fig. 2) as the average photosynthetic rate of the bracts and capsule wall, and multiply each by the respective area, the O_2 evolution capacity of the bolls was about 23.7% of the total at the full bolling stage (Table 2).

We assumed that 1,200 μmol photons m^{-2} s^{-1}, the irradiance under which the O_2 evolution rate was measured, was the saturation irradiance for all the green organs. This seems valid for two reasons. First, this irradiance was similar to the saturation irradiance for leaves (1,292 μmol m^{-2} s^{-1}), and higher than the saturation irradiance for bracts (344 μmol m^{-2} s^{-1}), main stem (344 μmol m^{-2} s^{-1}) and capsule wall (536 μmol m^{-2} s^{-1}), as obtained from light-response curves of ETR, the linear electron transport rate estimated from Chl fluorescence. The lower saturation irradiance in non-foliar organs should be robust because it is supported by the lower RuBPC activity in non-foliar organs (Fig. 3a). Second, the irradiance was above 1,200 μmol photons m^{-2} s^{-1} for 8.5 h in Xinjiang on a sunny day in summer (Fig. 6); the non-foliar organs would have been approximately light-saturated in the field despite their non-optimal orientation towards the light. We found that the photosynthetic rate of carpopodium was very similar to the main stem in 2008 (data not shown), so we only measured the main stem in 2009 and 2010. Therefore, as an approximation, we considered the photosynthetic rate of the main stem to be equal to that of the stalks (including carpopodium and petiole). It should be noted that this study examined only

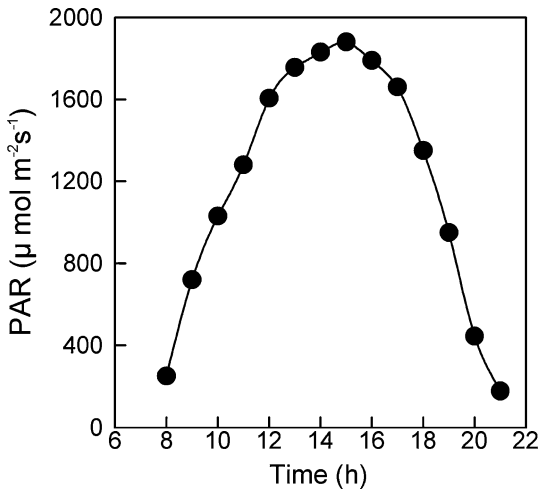

Fig. 6 Typical diurnal time-course of irradiance about 1 m above the ground from horizontal orientation

the main leaf and other non-foliar green organs. One limitation of this study was that we did not measure the O_2 evolution rate of subtending leaves of the corresponding bolls. Although we could have over-estimated the total photosynthetic capacity of the various green organs in the whole plant, this study does provide a useful assessment of the relative contribution of the different green organs to the photosynthesis of the whole plant.

Darkening the bolls and main stem had a significant effect on the boll weight per plant, 24.1 and 9%, respectively (Table 3). These results indicated that the calculation of the relative contribution of each organ to the whole plant was fairly accurate in Table 2. However, the effect of darkening bolls was larger than the estimation by Wullscheger et al. (1991) in which the capsule wall contributed approximate 10% of the carbon necessary for fruit dry weight during fruit development, based on measurement of the CO_2 re-fixation rate of the boll under light. Compared with the leaves, the capsule wall would be a greater source of assimilates because of its delayed senescence and stable photosynthetic rate at the late growth stage. Besides, the contribution of non-foliar organs to the reproductive biomass might have been enhanced because of the proximity of the source (capsule wall and bract) to the sink (fibre and seeds). The assimilates of non-foliar organs, especially the capsule wall, are probably destined mainly for the formation of fibre and seeds. In Xinjiang, the temperature at the late stage was lower than that at the peaking flowering stage, especially at night. A lower temperature would slow the translocation of assimilates at the plant level (Caldwell et al. 1977). Therefore, the capsule wall with short transport path to the fibre and seeds has a further advantage in nourishing the developing fibre and the filling seeds through its own photosynthate. This suggests that the reproductive biomass is largely affected by the photosynthesis at the late growth stage, when the photosynthetic contributions of non-foliar organs are larger than at previous stages. When we select high yield cotton cultivars, therefore, the photosynthetic capacity of non-foliar organs should also be taken into consideration. In future, further research should be conducted to improve the photosynthetic capacity of not only leaves but also of non-foliar organs to improve the yield of reproductive biomass.

In conclusion, the photosynthesis of non-foliar organs in cotton made a significant contribution to the yield, especially at the late growth stage. Compared with the peak flowering stage, the surface area of leaves decreased by 6.6% due to defoliation at the late growth stage, while the surface area of fruits (bract plus capsule wall) increased by 97.7%. Moreover, bracts and capsule wall showed less ontogenetic decrease in O_2 evolution capacity and photosynthetic enzyme activity than leaves at the late growth stage. Thus, the relative photosynthetic contribution of the non-foliar organs to the whole-plant was likely to increase at the late growth stage. In addition, darkening the bolls and main stem reduced the boll weight per plant by 24.1 and 9%, respectively. These results suggest that reproductive biomass is largely affected by photosynthesis of non-foliar organs, because reproductive biomass relies on the photosynthesis at the late growth stage when the photosynthetic contribution of non-foliar organs is remarkably increased from the previous stages.

Acknowledgments We thank XP Yi, XJ Zhang, T Liu, MS Yang and XB Hu for their support in plant culture and experiment and Jan Anderson for her helpful comments on the manuscript. This study was financially supported by a China Scholarship Council fellowship, the National Natural Science Foundation of China (Grant No. 31060176), the Special Launching Funds for High-level Talents of Shihezi University (Grant No. RCZX201005), JSPS Postdoctoral Fellowships for Research Abroad (21-674) and by the Australian Research Council (DP1093827).

References

Araus JL, Brown HR, Febrero A, Bort J, Serret MD (1993) Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO_2 to differences in grain mass in durum wheat. Plant Cell Environ 16:383–392

Aschan G, Pfanz H (2003) Non-foliar photosynthesis—a strategy of additional carbon acquisition. Flora 198:81–97

Aschan G, Pfanz H, Vodnik D, Batič F (2005) Photosynthetic performance of vegetative and reproductive structures of green hellebore (*Helleborus viridis* L. agg.). Photosynthetica 43:55–64

Aschan G, Pfanz (2006) Why snowdrop (*Galanthus nivalis* L.) tepals have green marks? Flora 201:623–632

Birkhold KB, Koch KE, Darnell RL (1992) Carbon and nitrogen economy of developing rabbiteye blueberry fruit. J Am Soc Hortic Sci 117:139–145

Blanke MM, Ebert G (1992) Phosphoenolpyruvate carboxylase and carbon economy of apple seedlings. J Exp Bot 43:965–968

Bondada BR, Oosterhuis DM, Wullschleger SD, Kim KS, Harris WM (1994) Anatomical considerations related to photosynthesis in cotton leaves, bracts, and the capsule wall. J Exp Bot 45:111–118

Bondada BR, Oosterhuis DM (2000) Comparative epidermal ultrastructure of cotton (*Gossypium hirsutum* L.) leaf, bract and capsule wall. Ann Bot 86:1143–1152

Bravd BA, Palgi A, Lurie S (1977) Changing ribulose diphosphate carboxylase/oxygenase activity in ripening tomato fruit. Plant Physiol 60:309–312

Caley CY, Duffus CM, Jeffcoat B (1990) Photosynthesis in the pericarp of developing wheat grains. J Exp Bot 41:303–307

Caldwell MM, Osmond CB, Nott DL (1977) C4 path photosynthesis at low temperature in cold-tolerant *Atriplex* species. Plant Physiol 60:157–164

Camp PJ, Huber SC, Burke JJ, Moreland DE (1982) Biochemical changes that occur during senescence of wheat leaves. I. Basis for the reduction of photosynthesis. Plant Physiol 70:1641–1646

Cockfield SD (1988) Relative availability of nitrogen in host plants of invertebrate herbivores: three possible nutritional and physiological definitions. Oecologia 77:91–94

Constable GA, Rawson HM (1980) Carbon production and utilization in cotton: inferences from a carbon budget. Aust J Plant Physiol 7:539–553

Du MW, Feng GY, Yao YD, Luo HH, Zhang YL, Xia DL, Zhang WF (2009) Canopy characteristics and its correlation with photosynthesis of super high-yielding hybrid cotton Biaoza A1 and Shiza 2. Acta Agron Sin 35:1068–1077(in Chinese with English abstract)

Duffus CM, Cochrane MP (1993) Formation of the barley grain-morphology, physiology, and biochemistry. In: MacGregor AW, Bhatty RS (eds) Barley: chemistry and technology. American Association of Cereal Chemists, St. Paul, pp 31–72

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9–19

Evans JR, Jakobsen I, Ögren E (1993) Photosynthetic light-response curves 2 Gradients of light-absorption and photosynthetic capacity. Planta 189:191–200

Evans JR (2009) Potential errors in electron transport rates calculated from chlorophyll fluorescence as revealed by a multilayer leaf model. Plant Cell Physiol 50:698–706

Field C, Mooney H (1986) The photosynthesis–nitrogen relationship in wild plants. In: Givnish GT (ed) On the economy of plant form and function. Cambridge University Press, Cambridge, pp 25–55

Hedley CL, Harvey DM, Keely RJ (1975) Role of PEP carboxylase during seed development in *Pisum sativum*. Nature 258:352–354

Ishihara K, Takada A, Imaizumi N (1991) On the contribution of panicle photosynthesis to grain yield in rice plants. Jpn J Crop Sci 60:122–123

Kingston-Smith AH, Harbinson J, Williams J, Foyer CH (1997) Effect of chilling on carbon assimilation, enzyme activation, and photosynthetic electron transport in the absence of photoinhibition in maize leaves. Plant Physiol 114:1039–1046

Li XJ, Wang HG, Li HB, Zhang LY, Teng NJ, Lin QQ, Wang J, Kuang TY, Li ZS, Li B, Zhang AM, Lin JX (2006) Awns play a dominant role in carbohydrate production during the grain-filling stages in wheat (*Triticum aestivum* L.). Physiol Plant 127:701–709

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382

Makino A, Mae T, Ohira K (1983) Photosynthesis and ribulose-1, 5-bisphosphate carboxylase in rice leaves. Plant Physiol 73:1002–1007

Makino A, Osmond B (1991) Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. Plant Physiol 96:355–362

Oguchi R, Douwstra P, Fujita T, Chow WS, Terashima I (2011) Intra-leaf gradients of photoinhibition induced by different color lights: implications for the dual mechanisms of photoinhibition and for the application of conventional chlorophyll fluorometers. New Phytol 191:146–159

Oosterhuis DM, Chipamaunga J, Bate GC (1983) Nitrogen uptake of field-grown cotton. I. Distribution in plant components in relation to fertilization and yield. Expl Agric 19:91–101

Prestidge RA, McNeill S (1983) The role of nitrogen in the ecology of grassland Auchenorryncha. In: Lee JA, McNeil S, Rorison IH (eds) Nitrogen as an ecological factor. Blackwell Scientific Publishers, Oxford, pp 257–281

Schuman GE, Stanley MA, Knudsen D (1972) Automated total nitrogen analysis of soil and plant samples. Proc Soil Sci Soc Am 37:480–481

Read SM, Northcote DH (1981) Minimization of variation in the response to different protein of the Coomassic Blue G dye binding assay for protein. Anal Biochem 116:53–64

Sayre RT, Kennedy RA, Pringnitz DJ (1979) Photosynthetic enzyme activities and localization in Mollugo verticillata populations differing in the levels of C_3 and C_4 cycle operation. Plant Physiol 64:293–299

Singal HR, Sheoran IS, Singh R (1986) In vitro enzyme activities and products of $^{14}CO_2$ assimilation in flag leaf and ear parts of wheat (*Triticum aestivum* L.). Photosynth Res 8:113–122

Tsuyama M, Shibata M, Kobayashi Y (2003) Leaf factors affecting the relationship between chlorophyll fluorescence and the rate of photosynthetic electron transport as determined from CO_2 uptake. J Plant Physiol 160:1131–1139

Xu HL, Gauthier L, Desjardins Y, Gosselin A (1997) Photosynthesis in leaves, fruits, stem and petioles of greenhouse-grown tomato plants. Photosynthetica 33:113–123

Wang ZM, Wei AL, Zheng DM (2001) Photosynthetic characteristics of non-foliar organs of winter wheat cultivars differing in ear type and their relationship with grain mass per ear. Photosynthetica 39:239–244

Walcroft AS, Whitehead D, Silvester WB, Kelliher FM (1997) The response of photosynthetic model parameters to temperature and nitrogen content in *Pinus radiata* D. Don Plant Cell Environ 20:1338–1348

Warren CR, Adams MA (2002) Phosphorus affects growth and partitioning of nitrogen to Rubisco in *Pinus pinaster*. Tree Physiol 22:11–19

Warren CR, Adams MA (2004) Evergreen trees do not maximize instantaneous photosynthesis. Trends Plant Sci 9:270–274

Wullschleger SD, Oosterhuis DM (1990) Photosynthetic and respiratory activities of fruiting forms within the cotton canopy. Plant Physiol 94:463–469

Wullschleger SD, Oosterhuis DM (1991) Photosynthesis, transpiration, and water-use efficiency of cotton leaves and fruit. Photosynthetica 25:505–515

Wullschleger SD, Oosterhuis DM, Hurren RG, Hanson PJ (1991) Evidence for light-dependent recycling of respired carbon dioxide by the cotton fruit1. Plant Physiol 97:574–579

Zhang YL, Feng GY, Hu YY, Yao YD, Zhang WF (2010) Photosynthetic activity and its correlation with matter production in non-foliar green organs of cotton. Acta Agron Sin 36:701–708 (in Chinese with English abstract)