

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

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23

1 **Abstract**

2 Non-foliar green organs are recognized as important carbon sources after leaves. However, the  
3 contribution of each organ to total yield has not been comprehensively studied in relation to the  
4 time-course of changes in surface area and photosynthetic activity of different organs at different  
5 growth stages. We studied the contribution of leaves, main stem, bracts and capsule walls in cotton by  
6 measuring their time-course of surface area development, O<sub>2</sub> evolution capacity and photosynthetic  
7 enzyme activity. Because of the early senescence of leaves, non-foliar organs increased their surface  
8 area up to 38.2% of total at late growth stage. Bracts and capsule wall showed less ontogenetic  
9 decrease in O<sub>2</sub> evolution capacity per area and photosynthetic enzyme activity than leaves at the late  
10 growth stage. The total capacity for O<sub>2</sub> evolution of stalks and bolls (bracts plus capsule wall) was  
11 12.7% and 23.7% (total *ca.* 36.4%), respectively, as estimated by multiplying their surface area by their  
12 O<sub>2</sub> evolution capacity per area. We also kept the bolls (from 15 days after anthesis) or main stem (at  
13 the early full bolling stage) in darkness for comparison with non-darkened controls. Darkening the  
14 bolls and main stem reduced the boll weight by 24.1% and 9%, respectively, and the seed weight by  
15 35.9% and 16.3%, respectively. We conclude that non-foliar organs significantly contribute to the  
16 yield at the late growth stage.

17 **Keywords:** Different growth stages · O<sub>2</sub> evolution · Photosynthetic enzymes · Surface  
18 area · Reproductive biomass

19

20 **Abbreviations**

21	ATP	Adenosine triphosphate
22	Chl	Chlorophyll
23	DAA	Days after anthesis
24	DTT	Dithiothreitol
25	EDTA	Ethylenediaminetetraacetate
26	MDH	Malate dehydrogenase
27	NADH	Reduced nicotinamide adenine dinucleotide

1	NADPH	Reduced nicotinamide adenine dinucleotide phosphate
2	PEPC	Phosphoenolpyruvate carboxylase
3	RuBPC	Ribulose 1,5-bisphosphate carboxylase

#### 4 **Introduction**

5 More than 90% of crop biomass is derived from photosynthetic products. Consideration and  
6 modeling of photosynthesis are usually restricted to leaves, but many parts of the plant besides leaves  
7 contain chlorophyll and, therefore, capture light energy to perform photosynthesis. Evidence of  
8 photosynthetic activity of non-foliar organs has been found in, for example, the panicles of rice  
9 (Ishihara et al. 1991), the ear of wheat (Singal et al. 1986; Araus et al. 1993; Li et al. 2006), the spikes  
10 of barley (Duffus and Cochrane 1993), and the fruit in tomato (Xu et al. 1997). It is now accepted that  
11 photosynthesis of non-foliar green organs is an important, additional contribution to carbon acquisition  
12 and yield (Aschan and Pfan 2003).

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

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

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

## 12 **Materials and methods**

### 13 Plant materials and growth conditions

14 Our study was conducted at an experimental field of Shihezi Agricultural College, Shihezi University,  
15 Xinjiang, China (45°19' N, 86°03' E) in 2009 and 2010. Cotton (*Gossypium hirsutum* L. cv. Xinluzao  
16 13) was grown under field conditions. Seeds were sown on 22 April in 2009 and 24 April in 2010 in  
17 rows 12 cm apart at a plant density of  $1.8 \times 10^5 \text{ ha}^{-1}$ . The plot was drip irrigated and maintained  
18 well-watered throughout the whole season. Pest and weed control was carried out according to the  
19 local standard practice. The experimental design was completely randomized with three replications.  
20 Materials for the measurements of O<sub>2</sub> evolution, photosynthetic enzymes and chlorophyll content were  
21 collected from the penultimate main leaves after tip pruning (July 5<sup>th</sup> in 2009 and July 10<sup>th</sup> in 2010), as  
22 well as from corresponding bolls, their associated bracts, and main stems under the main-stem leaves.  
23 To minimize age-related variability in the measured parameters, approximately 400 first position fruits  
24 at penultimate main stem leaves were labeled with plastic tags at anthesis (began July 25<sup>th</sup> in 2009 and  
25 July 10<sup>th</sup> in 2010). Ambient photosynthetically active irradiance was measured with an external  
26 quantum sensor attached to an infrared gas exchange analyzer (Li-6400; Li-Cor Inc., Lincoln, NE, USA)  
27 by positioning the sensor horizontal to the ground on a sunny day in July.

## 1 Surface area and biomass

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

## 13 Nitrogen content of all green organs

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

## 17 Measurements of photosynthetic O<sub>2</sub> evolution

18 The leaves, bracts, bolls and the main stem under the main leaf were removed from the plant at 09:30  
19 am, placed on moistened cloth, and then taken to the lab as quickly as possible. Samples of leaves,  
20 bracts, main stem and capsule wall were cut into small sections. Oxygen evolution capacity of the  
21 sectioned samples was measured at 25°C using a ChloroLab2 liquid-phase O<sub>2</sub> electrode system  
22 (Hansatech Instruments, Norfolk, UK), in which the CO<sub>2</sub> was saturating for photosynthesis. The  
23 reaction mixture (2.0 mL) was composed of 20 mM NaHCO<sub>3</sub> and 60 mM Tris-HCl (pH 7.5).  
24 Illumination (1200  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) was provided by red light-emitting diodes. The area and  
25 fresh weight of leaves, bracts, main stem and capsule wall were also recorded at each measurement  
26 time point. All the O<sub>2</sub> evolution capacities were calculated on an area basis ( $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and

1 fresh weight basis ( $\mu\text{mol O}_2 \text{ g FW}^{-1} \text{ min}^{-1}$ ). Data were averaged from six replicates.

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

3 The key photosynthetic enzymes of leaves, bolls, bracts and main stems were investigated on 5, 20 and  
4 50 DAA. The extraction of enzymes was carried out according to Sayre et al. (1979) with slight  
5 modifications. Green tissue (0.2 g) was ground with a mortar and pestle ( $4^\circ\text{C}$ ) containing a small  
6 amount of sand and 1.0 mL of grinding media consisting of 0.1 M Tris-HCl (pH 7.8), 100 mM  $\text{MgCl}_2$ ,  
7 1 mM EDTA, 20 mM mercaptoethanol, 100  $\text{kg}\cdot\text{m}^{-3}$  glycerin, and 10  $\text{kg}\cdot\text{m}^{-3}$  polyvinylpyrrolidone.  
8 Following centrifugation at 15,000  $g$  for 10 min at  $4^\circ\text{C}$ , the supernatant was used for enzyme assays.  
9 RuBPC activity was assayed by the method of Camp et al. (1982). An enzyme extract was added to a  
10 reaction mixture that contained 50 mM Tricine-NaOH (pH 7.9), 10 mM KCl, 1 mM EDTA, 2 mM  
11 dithiothreitol (DTT), 0.2 mM NADH, 5 mM ATP, 15 mM  $\text{MgCl}_2$ , 10 mM  $\text{NaHCO}_3$ , 5 mM  
12 phosphocreatine, 2 units per mL creatine phosphokinase, 4 units per mL each of NAD-dependent  
13 glyceraldehyde-3-P dehydrogenase and 3-P-glycerate kinase in a final volume of 1 mL. The mixture  
14 was incubated at  $25^\circ\text{C}$  for 5 min. Reactions were initiated by the addition of 0.5 mM RuBP.

15 For PEPC activity assay, about 0.2 g of each sample was frozen in liquid nitrogen and pulverized  
16 with a mortar and 1.0 mL of grinding medium consisting of 1 mM Tris- $\text{H}_2\text{SO}_4$  (pH 8.2), 7 mM  
17 mercaptoethanol, 1 mM EDTA, 5% (v/v) glycerol, and 1% (w/v) insoluble polyvinylpyrrolidone. The  
18 homogenates were filtered through cheesecloth, and the filtrates were centrifuged at 20,000  $g$  for 15  
19 min at  $4^\circ\text{C}$ . The supernatants were used for assays of enzymatic activity. PEPC activity was  
20 measured spectrophotometrically at 340 nm using a spectrophotometer (U-3900, Hitachi, Japan) and by  
21 coupling the PEP reaction to the oxidation of NADH with malate dehydrogenase (MDH) according to  
22 Blanke and Ebert (1992). The enzyme extract was added to a solution which, in a 1.5 mL final  
23 volume contained 50 mM Tris-HCl (pH 7.8), 10 mM  $\text{MgCl}_2$ , 0.25 mM EDTA, 5.0 mM  $\text{NaHCO}_3$ , 2.0  
24 mM DTT, 4 unit MDH, 0.1 mM NADH, and 2.0 mM PEP. The reaction was started by the addition of  
25 tissue extract. For consistency with the oxygen evolution rate, each enzyme activity was calculated  
26 on both an area basis and fresh weight basis.

27 The soluble protein content of leaves was measured according to the Coomassie brilliant blue  $\text{G}_{250}$

1 method described by Read and Northcote (1981). The soluble protein content was measured by a  
2 spectrophotometer at 595 nm.

3 Chlorophyll content of all green organs

4 The content of chlorophyll of plant organs was determined in discs from leaves, bracts or capsule wall  
5 tissue removed by a calibrated metallic borer (4 mm diameter), or a main stem segment of known size.

6 Discs of the green organs were extracted in 80% (v/v) acetone for 24 h at room temperature in the dark.

7 The absorbance of an extract was measured with a spectrophotometer, and the chlorophyll content was  
8 calculated according to Lichtenthaler (1987).

9 The total capacity for O<sub>2</sub> evolution of the green organs to the whole plant

10 The contribution of photosynthesis in each green organ to the whole plant was calculated as the product  
11 of the surface area and the O<sub>2</sub> evolution rate per area (Fig. 1a, c, and Fig. 2a, b, respectively), at the  
12 peak flowering stage and the late full bolling stage (Table 2). For the calculation of the peak  
13 flowering stage (July 17<sup>th</sup>), since the surface area of the capsule wall at 5 DAA was too small for  
14 measurement of photosynthesis rate, the O<sub>2</sub> evolution rate of capsule wall was chosen as that at 15  
15 DAA, while the average value of 5 DAA and 15 DAA was taken as the average photosynthetic rate of  
16 the other organs. We used the instantaneous area at the peak flowering stage, shown in Fig. 1a, for the  
17 surface area. For the calculation of the late growth stage (August 22<sup>nd</sup>), when the bolls in different  
18 parts of the plant were at different growth stages, the average value from 20 DAA to 50 DAA was taken  
19 as the average photosynthetic capacity of each organ. The instantaneous area at the full bolling stage,  
20 which was shown in Fig. 1c, was used as the surface area. The percent contribution of photosynthesis  
21 in each green organ to the total plant was estimated by the total photosynthesis rate of each organ  
22 divided the photosynthesis rate of the whole plant (the sum of all organs).

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

24 From 15 days after emergence of each boll to harvest, the bolls (capsule wall plus bracts) of nine plants  
25 were kept in the dark by covering with aluminium foil, following the method of Araus et al. (1993).

26 Similarly, the main stem of nine other randomly selected plants were kept in the dark from 1<sup>st</sup> August,

1 about a fortnight after peak flowering. Nine other non-darkened plants were tagged at this point.  
2 The dry capsule wall weight per boll, seed weight per boll and fibre weight per plant were measured for  
3 the control, boll-darkened, and main stem-darkened plants (on 27<sup>th</sup> September). Although darkening  
4 by covering the organ with aluminium may not only affect the light intensity but also the temperature  
5 and composition of the air around the organ, it is a common method to assess the relative contribution  
6 of the green organs photosynthesis to the plant. In our study, we attempted to limit the build-up of  
7 ethylene and water vapour around the non-foliar organs by having small holes (at least 15 mm apart,  
8 and representing about 0.3% of the covered area) in the aluminium foil.

9 
$$\text{Relative contribution (\%)} = 100 \times (\text{control yield} - \text{darkened yield}) / \text{control yield}$$

## 10 Data analysis

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

## 15 Results

### 16 Surface area and biomass of green organs

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

### 24 O<sub>2</sub> evolution capacity

25 On an area basis, the rate of oxygen evolution in main leaves showed a pronounced decrease during the



1 development of bolls (Fig. 2a, c). By comparison, bracts reached an obvious peak in photosynthesis  
2 ( $5.0\text{-}5.2 \mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) at about 15 d after anthesis, which was about 42-44% of that of the main  
3 leaves at 15 DAA (Fig. 2a). Beyond this time, bracts followed a declining trend with age. The main  
4 stem maintained higher  $\text{O}_2$  evolution rates than bracts during ontogeny, but the  $\text{O}_2$  evolution rate  
5 decreased monotonically with age. The  $\text{O}_2$  evolution rate of capsule wall on an area basis was higher  
6 than that of leaves during the late stage of boll development, having peaked at 20 DAA.

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

#### 11 Photosynthetic enzymes and soluble protein content at different growth stages

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

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

#### 27 Nitrogen content of leaves, bracts and capsule wall

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

#### 5 Chlorophyll analyses

6 Both the Chl content, based on the area or fresh weight analyses, of non-foliar organs (bracts, main  
7 stem and capsule wall) were significantly lower than those of leaves (Fig. 5a, b). On a surface area  
8 basis, the Chl content in leaves decreased with age. However, the Chl content in non-foliar organs  
9 slightly increased in the beginning, followed by a small decrease with age (Fig. 5a). On a fresh  
10 weight basis, the decline in the Chl content was less in non-foliar organs than in leaves (Fig. 5c, d).

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

12 The oxygen evolution rate can be an indication of photosynthetic activity (Caley et al.1990). Based  
13 on the mean surface area and the O<sub>2</sub> evolution rate of each Chl-containing organ during the growth  
14 stages (Fig. 1, 2), the relative photosynthetic contribution of the different parts to the total plant in  
15 terms of carbon assimilation was estimated by multiplying the O<sub>2</sub> evolution rate per area by the total  
16 area of the respective organ (Table 2), following the method of Aschan et al. (2005) who used the CO<sub>2</sub>  
17 exchange rate. As shown in Table 2, the photosynthetic contribution of stalks and the bolls (bracts  
18 plus capsule wall) were 9.7% and 4.4% of the total, respectively, at the peak flowering stage. At the  
19 late full bolling stage, the total photosynthetic contribution of the stalks and bolls were 12.7% and  
20 23.7%, respectively (Table 2). Overall, the non-foliar green organs contributed about 14% and 36.4%  
21 of photosynthesis to the whole plant at the peak flowering stage and full bolling stage, respectively.

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

23 The relative photosynthetic contribution of bolls (capsule walls plus bracts) and the main stem to boll  
24 dry weight, seed dry weight, lint dry weight and capsule dry weight per plant was assessed by the  
25 reduction of their weight by darkening bolls or the main stem (Table 3). The plants with darkening of  
26 the bolls had significantly reduced boll number per plant compared to non-darkened plants. Boll

1 weight was reduced by the darkening of bolls and the main stem, by 24.1% and 9%, respectively.  
2 Seed weight per plant was reduced by the darkening of bolls and the main stem, by 35.9% and 16.3%,  
3 respectively. The contribution of bolls to fibre was about 8.8%. The bolls contributed 25.1% of the  
4 dry weight in the capsule wall, while the main stem contributed 5.4%.

## 5 **Discussion**

6 Differences in O<sub>2</sub> evolution rate and carboxylation enzymes of different green organs in cotton

7 The photosynthetic performance of the non-foliar organs is more and more studied by the chlorophyll  
8 (Chl) fluorescence techniques (Ashan and Pfanz 2003; Aschan et al. 2005; Aschan and Pfanz 2006).  
9 However, chlorophyll fluorescence data do not necessarily represent the photosynthetic status of the  
10 whole leaf tissue, especially in thick leaves, a deficiency which has been shown by close comparisons  
11 of the electron transport rates measured with chlorophyll fluorescence and with gas-exchange  
12 (Kingston-Smith et al. 1997; Tsuyama et al. 2003). The measuring light intensity diminishes with  
13 increasing depth in the tissue, and the possibility of re-absorption of the chlorophyll fluorescence signal  
14 is increased for the chlorophyll fluorescence emitted in the deeper tissue (Evans et al. 1993; Evans,  
15 2009). Oguchi et al. (2011) measured photoinhibited leaves with various conventional fluorometers,  
16 and concluded that fluorometers with red-measuring light probe deeper into leaf tissue than those with  
17 blue-measuring light. Bondada et al. (1994) have found that the capsule wall (1013 µm) was much  
18 thicker compared with the leaves (152 µm) in cotton (*Gossypium hirsutum* L.). Accordingly, although  
19 PSII quantum yield in the capsule wall was estimated as 33%-64% of that in leaves with the  
20 chlorophyll fluorescence techniques (Zhang et al. 2009), their conclusion may be subjected to some  
21 uncertainties. Hence, we aimed to measure the photosynthetic capacity of the whole tissue by using a  
22 liquid-phase oxygen electrode.

23 The capacity for oxygen evolution on an area basis in our study was substantially higher in the  
24 capsule wall than that in leaves at 20 DAA (Fig. 2). At about 20 DAA, the N content per area was  
25 also significantly (about 4.2 fold) higher in the capsule wall than in leaves (Table 1). Our findings are  
26 consistent with those of Wullschleger and Oosterhuis (1990). More than half of the leaf nitrogen is  
27 allocated to photosynthetic proteins (Evans 1989; Makino and Osmond 1991), such as Calvin-Benson

1 cycle enzymes, coupling factor and electron carriers; these proteins are important in determining the  
2 photosynthetic capacity, which generally strongly correlates with the total amount of leaf nitrogen per  
3 unit leaf area (Field and Mooney 1986; Evans 1989; Walcroft et al. 1997). Because nitrogen content  
4 on a dry weight basis (%) of the capsule wall was significantly lower than that of leaves, the higher  
5 nitrogen content per area was due to the much greater thickness of capsule wall than leaves. Thus, we  
6 tentatively attribute the higher oxygen evolution rate of capsule wall per area to its greater thickness.

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

21 There is another possible reason for the RuBPC per area being lower in the capsule wall compared  
22 with the leaves, while the soluble protein content per area was higher in the capsule wall. Some of the  
23 RuBPC, together with other soluble proteins, might merely serve to store nitrogen in the form of  
24 protein, without being fully active (Warren and Adams 2004). Even in leaves, proteins are probably  
25 better nitrogen stores than amino acids because the latter may increase the attractiveness of foliage for  
26 insect herbivores (Prestidge and McNeill 1983; Cockfield 1988). Warren and Adams (2002)  
27 suggested that the over-investment in Rubisco by *Pinus pinaster* is an adaptation to temporal variation  
28 in N supply which may help growth and photosynthesis during periods of N deficiency. Similarly, we  
29 suggest that the abundant soluble protein in capsule wall provides a more metabolically compatible

1 means of N storage that is less vulnerable to insect attack than leaves, thereby serving as a useful  
2 nutrient supply when needed. To test this hypothesis, further studies are necessary to explore whether  
3 the capsule wall is less susceptible in nitrogen deficiency.

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

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

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

24 According to a previous study, maximum net photosynthesis rate in cotton leaves is achieved  
25 about 15 days after unfolding, but photosynthesis is maintained at this rate for only 12 days, after  
26 which the rate of net photosynthesis declines, reaching near zero because of leaf senescence by about  
27 70 days (Constable and Rawson 1980). A short time before flowering, subtending leaves start to  
28 emerge. According to Wullschleger and Oosterhuis (1990), the subtending leaf at the time of anthesis

1 is 18 days old at the 8<sup>th</sup> node on the main-stem. Similarly, we also usually found that the subtending  
2 leaf was about 20 days old at the time of anthesis. Oosterhuis et al. (1983) suggested that the nitrogen  
3 content of main stem leaves and subtending leaves begin to decrease at about 30 days and 70 days from  
4 sowing, respectively. On the other hand, the photosynthetic rate of the capsule wall and bracts was  
5 largely maintained after flowering (Fig. 2). When main leaves begin to senesce after the bolls emerge,  
6 the major photosynthetic role needs to be assumed by the young subtending leaves, and other non-foliar  
7 organs. Thus, we suggest that the dramatically increased surface area of the bracts and the capsule  
8 wall, both of which maintained their photosynthesis rates for a longer period than leaves, would  
9 compensate for the loss of leaf photosynthetic activity at the late full bolling stage.

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

17 Chlorophyll plays an important role in the light absorption and energy transduction, which is the  
18 basis of photosynthesis. In our study, the decrease in Chl content of non-foliar organs was generally  
19 less than that of leaves (Fig. 5). Degradation of photosynthetic pigments is a typical characteristic of  
20 leaf senescence. The smaller decrease in O<sub>2</sub> evolution rate and Chl content in non-foliar organs  
21 indicates slower senescence than in leaves at the late growth stage.

## 22 Contribution of photosynthesis in non-foliar green organs to yield

23 There are several physiological studies of photosynthesis in non-foliar organs in cotton (Wullschleger  
24 and Oosterhuis 1990; Bondada et al. 1994; Bondada and Oosterhuis 2000); however, the contribution  
25 of non-foliar organs to yield is still not well quantified. In blueberry fruit (*Vaccinium ashei* Reade),  
26 Birkhold et al. (1992) estimated that photosynthesis contributes about 15% of the total carbohydrate  
27 required for fruit growth. In rice, 20-30% of the carbohydrates accumulated in the grains are derived  
28 from CO<sub>2</sub> assimilated in the panicles after their emergence (Ishihara et al. 1991). The total

1 contribution of non-foliar green organs, including ears and peduncles, accounts for about 40-50% of  
2 grain mass per ear (Araus et al. 1993; Wang et al. 2001). Indeed, the physiological importance of  
3 photosynthesis of green stems and other green parts in cotton cannot be neglected, especially at the late  
4 stage of growth.

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

18 We assumed that 1200 μmol photons m<sup>-2</sup> s<sup>-1</sup>, the irradiance under which the O<sub>2</sub> evolution rate was  
19 measured, as the saturation irradiance for all the green organs. This seems valid for two reasons.  
20 First, this irradiance was similar to the saturation irradiance for leaves (1292 μmol m<sup>-2</sup> s<sup>-1</sup>), and higher  
21 than the saturation irradiance for bracts (344 μmol m<sup>-2</sup> s<sup>-1</sup>), main stem (344 μmol m<sup>-2</sup> s<sup>-1</sup>) and capsule  
22 wall (536 μmol m<sup>-2</sup> s<sup>-1</sup>), as obtained from a light-response curves of ETR, the linear electron transport  
23 rate estimated from Chl fluorescence. **The lower saturation irradiance in non-foliar organs should be**  
24 **robust because it is supported by the lower RuBPC activity in non-foliar organs (Fig. 3a).** Second, the  
25 irradiance was above 1200 μmol photons·m<sup>-2</sup>·s<sup>-1</sup> for 8.5 hours in Xinjiang on a sunny day in summer  
26 (Fig. 6); the non-foliar organs would have been approximately light-saturated in the field despite their  
27 non-optimal orientation towards the light. We found the photosynthetic rate of carpopodium was very  
28 similar to the main stem in 2008 (data not shown), so we only measured the main stem in 2009 and  
29 2010. Therefore, as an approximation, we considered the photosynthetic rate of the main stem to be

1 equal to that of the stalks (including carpodium and petiole). It should be noted that this study  
2 examined only the main leaf and other non-foliar green organs. One limitation of this study was that  
3 we did not measure the O<sub>2</sub> evolution rate of subtending leaves of the corresponding bolls. Although  
4 we could have over-estimated the total photosynthetic capacity of the various green organs in the whole  
5 plant, this study does provide a useful assessment of the relative contribution of the different green  
6 organs to the photosynthesis of the whole plant.

7 Darkening the bolls and main stem had a significant effect on the boll weight per plant, 24.1% and  
8 9%, respectively (Table 3). These results indicated that the calculation of the relative contribution of  
9 each organ to the whole plant was fairly accurate in Table 2. However, the effect of darkening bolls  
10 was larger than the estimation by Wullscheger et al. (1991) in which the capsule wall contributed  
11 approximate 10% of the carbon necessary for fruit dry weight during fruit development, based on  
12 measurement of the CO<sub>2</sub> re-fixation rate of the boll under light. Compared with the leaves, the  
13 capsule wall would be a greater source of assimilates because of its delayed senescence and stable  
14 photosynthetic rate at the late growth stage. Besides, the contribution of non-foliar organs to the  
15 reproductive biomass might have been enhanced because of the proximity of the source (capsule wall  
16 and bract) to the sink (fibre and seeds). The assimilates of non-foliar organs, especially the capsule  
17 wall, are probably destined mainly for the formation of fibre and seeds. In Xinjiang, the temperature  
18 at the late stage was lower than that at the peaking flowering stage, especially at night. A lower  
19 temperature would slow the translocation of assimilates at the plant level (Caldwell 1977). Therefore,  
20 the capsule wall with short transport path to the fibre and seeds has a further advantage in nourishing  
21 the developing fibre and the filling seeds through its own photosynthate. This suggests that the  
22 reproductive biomass is largely affected by the photosynthesis at the late growth stage, when the  
23 photosynthetic contributions of non-foliar organs are larger than at previous stages. When we select  
24 high yield cotton cultivars, therefore, the photosynthetic capacity of non-foliar organs should also be  
25 taken into consideration. In future, further research should be conducted to improve the  
26 photosynthetic capacity of not only leaves but also of non-foliar organs to improve the yield of  
27 reproductive biomass.

28 In conclusion, the photosynthesis of non-foliar organs in cotton made a significant contribution to  
29 the yield, especially at the late growth stage. Compared with the peak flowering stage, the surface



1 area of leaves decreased by 6.6% due to defoliation at the late growth stage, while the surface area of  
2 fruits (bract plus capsule wall) increased by 97.7%. Moreover, bracts and capsule wall showed less  
3 ontogenetic decrease in O<sub>2</sub> evolution capacity and photosynthetic enzyme activity than leaves at the  
4 late growth stage. Thus, the relative photosynthetic contribution of the non-foliar organs to the  
5 whole-plant was likely to increase at the late growth stage. In addition, darkening the bolls and main  
6 stem reduced the boll weight per plant by 24.1% and 9%, respectively. These results suggest that  
7 reproductive biomass is largely affected by photosynthesis of non-foliar organs, because reproductive  
8 biomass relies on the photosynthesis at the late growth stage when the photosynthetic contribution of  
9 non-foliar organs is remarkably increased from the previous stages.

10

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- 5

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

4  
5 **Fig. 2** CO<sub>2</sub>-saturated oxygen evolution rates of leaves, bracts, main stem and capsule wall expressed on  
6 both surface area (**a, b**) and on fresh weight (**c, d**) bases at various days after anthesis in 2009 and 2010.  
7 Measurements were made at an irradiance of 1200 μmol photon m<sup>-2</sup>.s<sup>-1</sup>

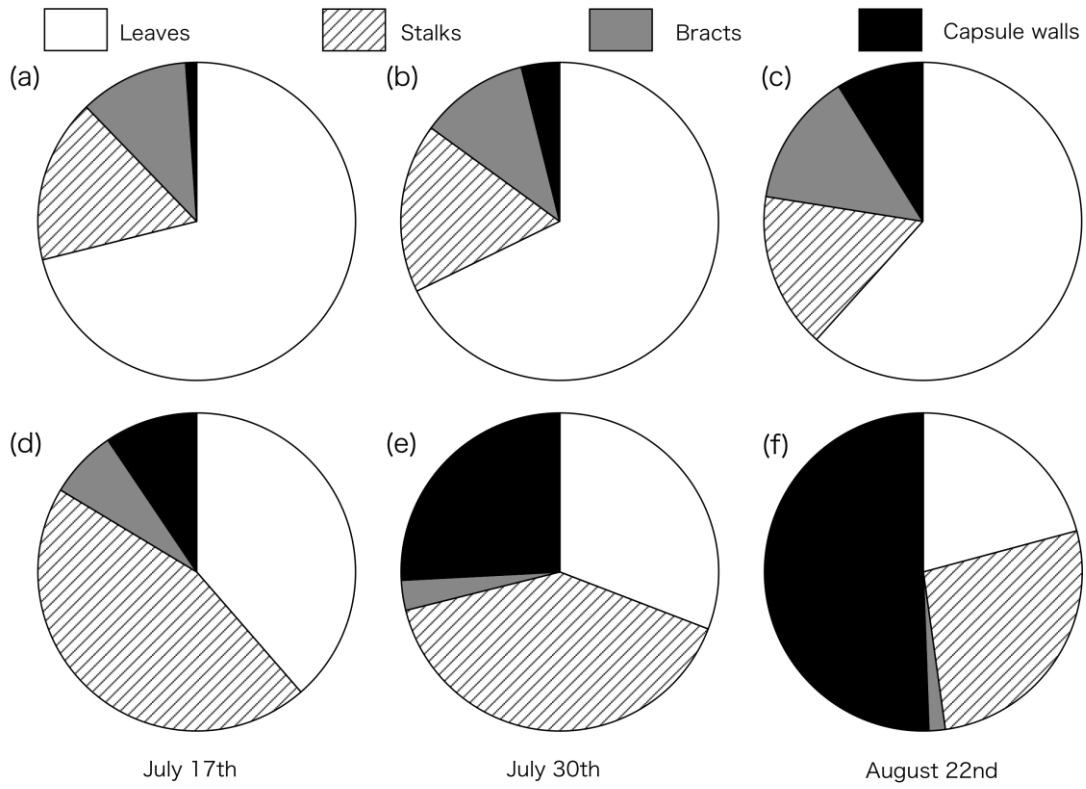
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9 **Fig. 3** Activities of Ribulose-1,5- biphosphate carboxylase (RuBPC) expressed on the basis of area (**a**)  
10 or fresh weight (**b**) in leaf and non-foliar green organs of cotton at various days after anthesis in 2010.  
11 Activities of phosphoenolpyruvate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

14  
15 **Fig. 4** Soluble protein content expressed on the basis of area (**a**) or fresh weight (**b**) in the leaf and  
16 non-foliar organs of cotton at various days after anthesis in 2010. Between organs, bars with the same  
17 letter above them are not significantly different ( $P \leq 0.05$ )

18  
19 **Fig. 5** Changes in total chlorophyll (Chl) content of leaves, bracts, main stem and capsule wall  
20 expressed on the basis of surface area (upper panels) or fresh weight (lower panels) during various  
21 times after anthesis in 2009 (**a, c**) and 2010 (**b, d**)

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23 **Fig. 6** Typical diurnal time-course of irradiance about 1 meter above the ground from **horizontal**  
24 **orientation**

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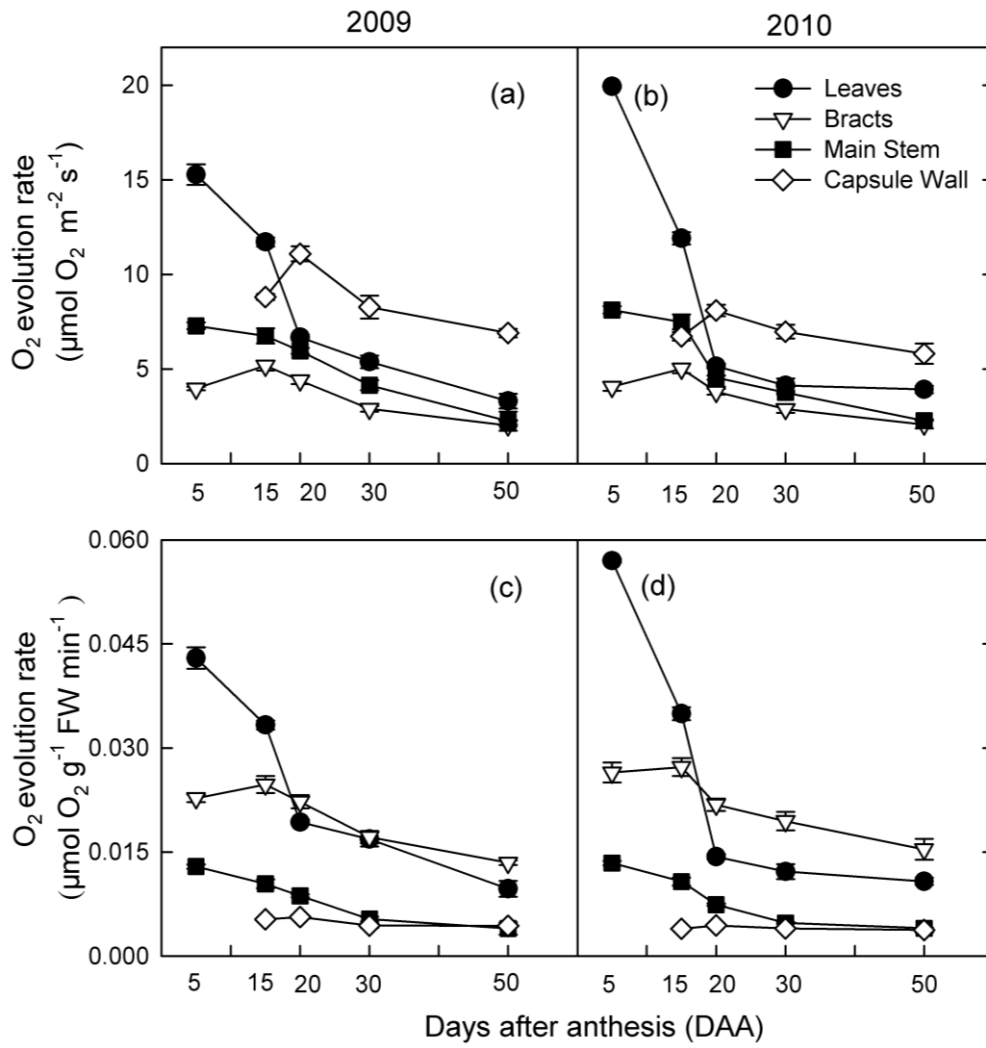


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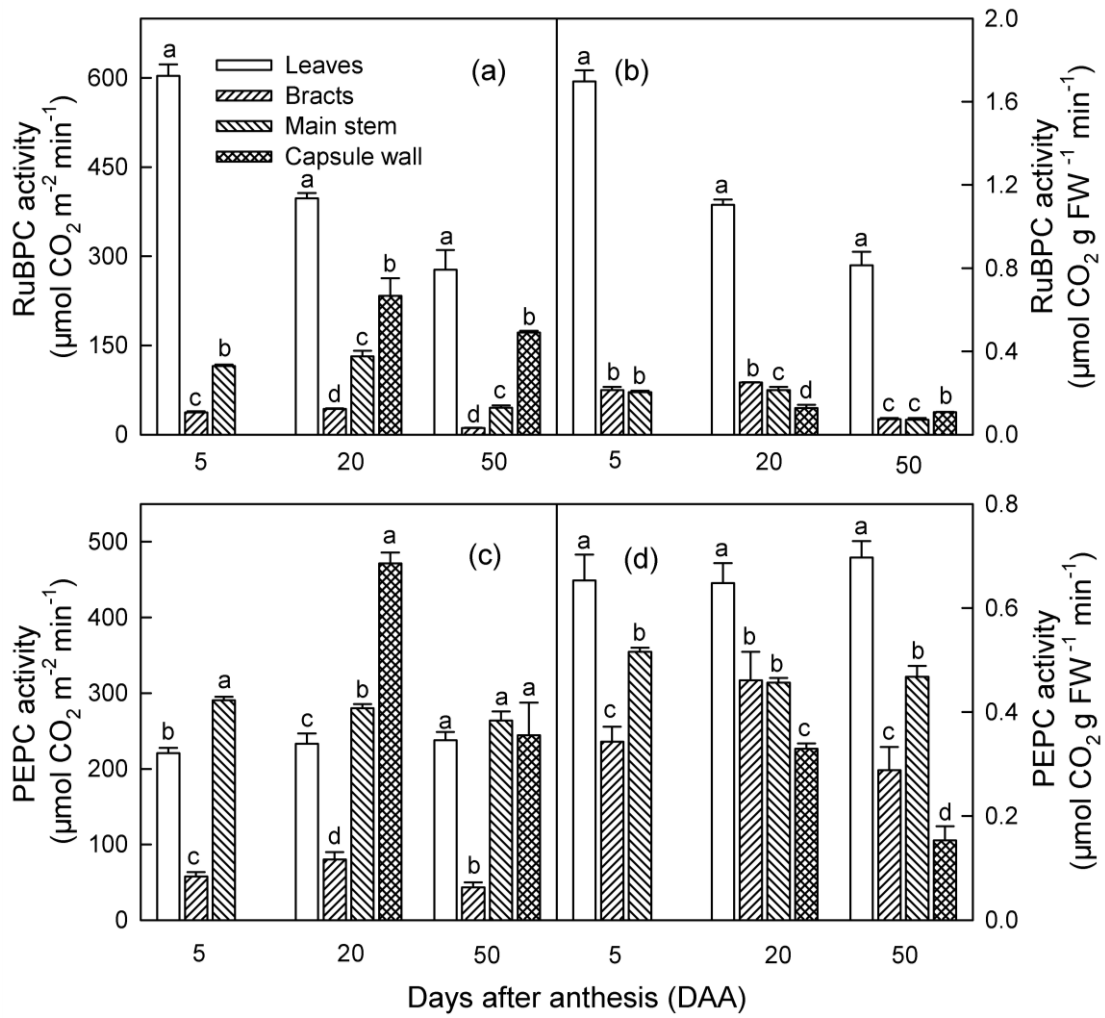
5 Fig. 2

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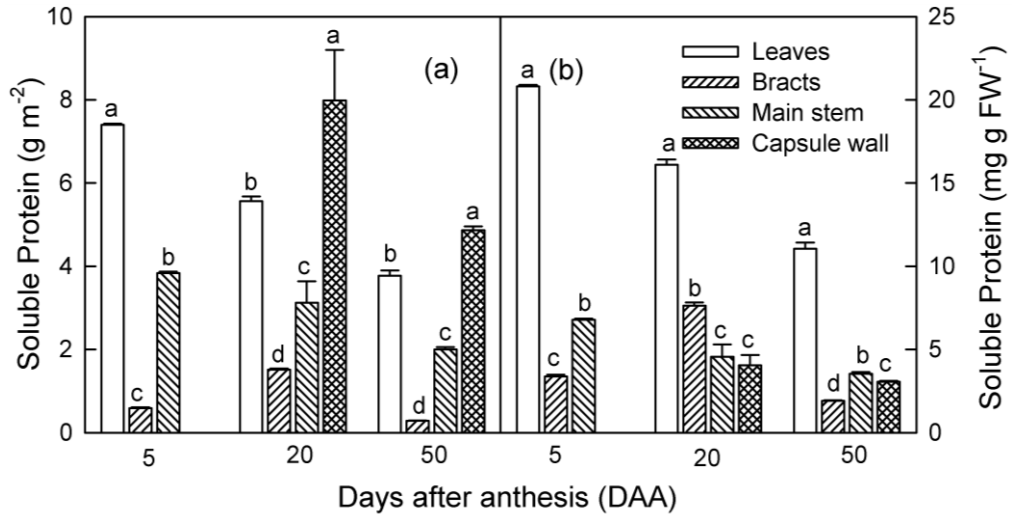
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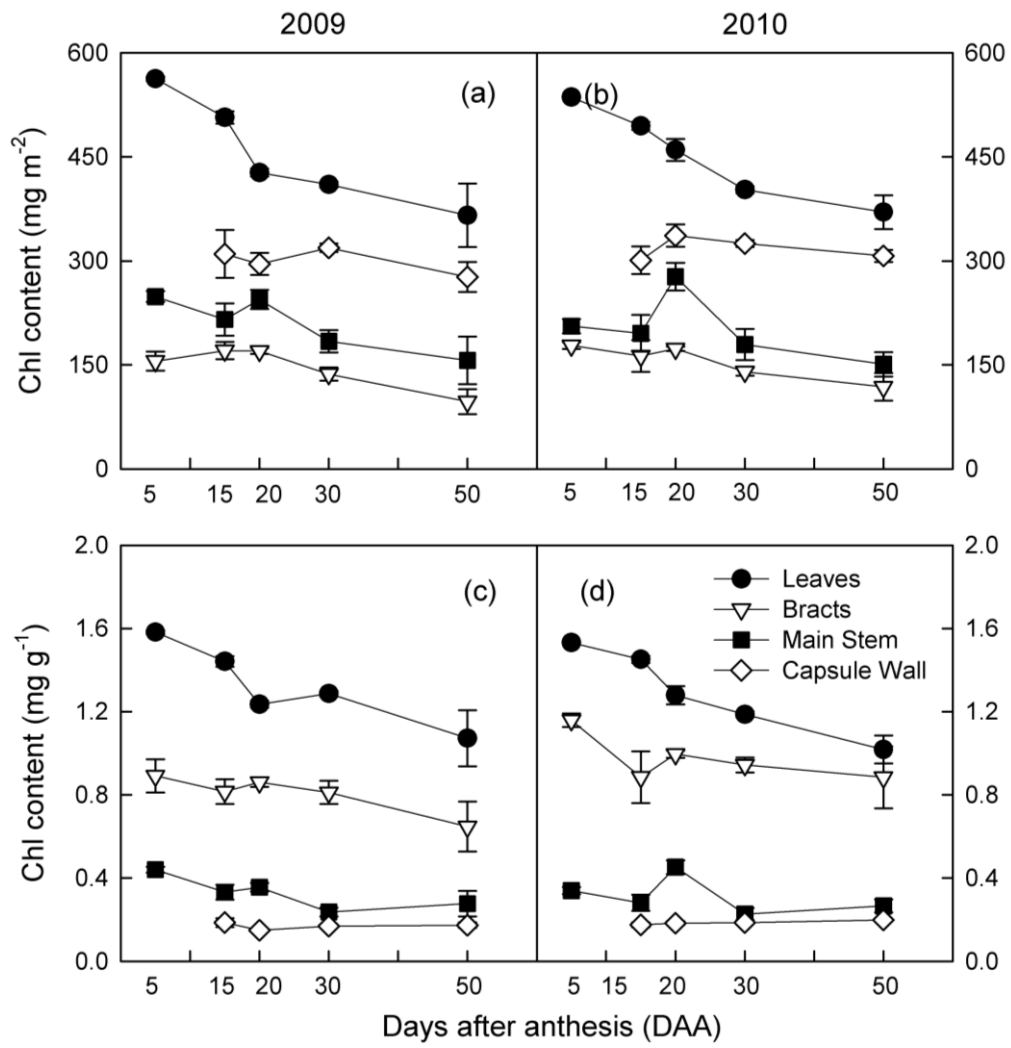
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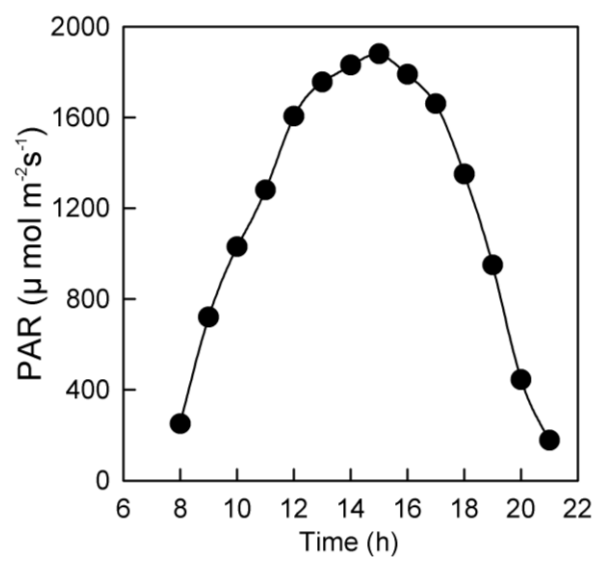
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