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Effect of shading on yield, fiber quality and physiological characteristics of cotton subtending leaves on different fruiting positions

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Abstract

Little is known regarding to impact of simulated shading conditions on cotton yield and fiber quality at different fruiting positions. In this 2-year study, our field experiments investigated the effects of shading percentage on the cotton yield, fiber properties, photosynthesis, and carbohydrate concentrations in boll's subtending leaves during various growing stages at different fruiting positions (FP). Net photosynthetic rate and effective quantum yield of PSII photochemistry decreased in response to shading on both FP1 and FP3 of the 7th sympodial branches, respectively. Shading also reduced sucrose and starch contents of leaves at each fruiting position. Shading decreased the number and mass of cotton bolls, the fiber strength and micronaire, while the fiber length increased at both fruiting positions. Our results suggested that shading resulted in the reduction of the cotton yield and fiber quality, which are mainly associated with the changes in boll number and alteration of photosynthesis and carbohydrate concentrations during the boll development.

Additional key words: chlorophyll fluorescence; cotton (*Gossypium hirsutum* L.); photosynthetically active radiation; position effect.

Introduction

The cotton yields are mainly produced by the cotton bolls at the fruiting position (FP) 1 and 2 of the sympodial branches (Jenkins *et al.* 1990, Oosterhuis 1990, Heitholt 1993, Anjum *et al.* 2001). In some areas with high seed cotton yields (7,657 kg ha^{-1}; 24,240 plants ha^{-1}), the boll retention rate on the distal sites (FP3 and greater) can reach 58.8% (Gu *et al.* 2010).

Photosynthesis is the base of crop growth and development, thus determining the crop yield (Pettigrew and Meredith 1994, Thompson *et al.* 1995, Zhao *et al.* 2004, Zeng *et al.* 2012). The development of cotton bolls depends primarily on subtending leaves (Ashley 1972, Kerby and Buxton 1981). Substantial alteration of photosynthesis has the potential to alter the crop growth. It is well documented that genotypic differences, biotic and abiotic resistances had significant effects on cotton leaf photosynthesis (Pettigrew and Turley 1998, Zhao and Oosterhuis 2000b, Pettigrew 2004, Chen *et al.* 2005). The photosynthesis of subtending leaves was limited by shading during flowering and boll formation stages (Bondada and Oosterhuis 2001, Pettigrew and Meredith 2012, Zhang *et al.* 2013). Therefore, studies on the leaves subtending to cotton boll is crucial to increase the fiber yield.

Cotton (*Gossypium hirsutum* L.) suffers from cloudy and rainy weather during the flowering and boll formation stage. The lint yield was affected by the amount of sunlight intercepted (Chen *et al.* 2014). Previous studies have shown that low irradiance decreases the yield, fiber micronaire, and fiber strength, while increases the shedding of squares and bolls in cotton (Pettigrew 1994, 1995; Zhao and Oosterhuis 1998a). Reduction of irradiance limits the carbon assimilation, which decreases the net

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Abbreviations: C_i – intercellular CO_2 concentration; Chl – chlorophyll; DM – dry mass; FP – fruit branch; F_v/F_m – quantum efficiency of PSII; g_s – stomatal conductance; LMA – leaf mass per area; MIC – fiber micronaire; P_N – net photosynthetic rate; Φ_{PSII} – effective quantum yield of PSII photochemistry; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; S0 – no shading, control; S1 – 21% of solar radiation blocked; S2 – 38% of solar radiation blocked; ST – fiber strength; TAA – time after anthesis; UHM – fiber length.

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photosynthetic rate, nonstructural carbohydrate concentrations, and chlorophyll (Chl) fluorescence in cotton and other plants (Pettigrew 1995, Zhao and Oosterhuis 2000a, Pettigrew 2001, 2004).

Light deficiency can decrease the cotton yield and fiber quality (Pettigrew 1994, 1995). However, little is known about its impact on different fruiting positions. The objective of this study was (*1*) to investigate the response of different fruiting positions to shading by measuring the photosynthesis and Chl fluorescence parameters, and carbohydrate profiles of subtending leaves. (*2*) to compare the yield, yield components, and fiber properties among shading treatments.

Materials and methods

Experimental design: Field experiments were performed at the Pailou Experimental Station of the Nanjing Agricultural University, Nanjing (32°02'N and 118°50'E), Jiangsu, China, in the Yangtze River Valley in 2010 and 2011. The soil at the experimental site was a alfisols (udalfs; FAO luvisol) with 18.3 and 16.5 g (organic matter) kg^{-1}, 1.1 and 1.0 g(total N) kg^{-1}, 73.2 and 80.5 mg(available N) kg^{-1}, 17.9 and 22.4 mg (available P) kg^{-1}, and 154.6 and 189.5 mg(available K) kg^{-1} contained in 0–20 cm depth of the soil profile in 2010 and 2011, respectively. Cotton (*Gossypium hirsutum* L.) cultivar was Sumian 15, which is a typical genotype planted widely in the Yangtze River Valley in China.

The treatments consisted of a unshaded control and two shading treatments in which cotton plants were covered with two different types of white polyethylene nets, without affecting the light quality. The shading treatments started at the flowering stage when 50% of the plants at the fruiting position 1 of the 6th sympodial branches have the white blooms, and ended at the boll-opening stage; it lasted for 48 d in 2010 and 46 d in 2011. The shading was realized with two types of net, which blocked 21% (S1) and 38% (S2) of the solar radiation above the canopy in clear days, respectively. No shading treatment was a control (S0). The nets were placed more than 2 m above the ground and sides in each plot. In order to have a good ventilation, both north and south sides open up the space of 10 cm. Cotton was sowed in a nursery bed on 25 April, and seedlings with three true leaves were transplanted to a field at final populations of 49,350 plants ha^{-1} in 2010 and 2011. Each plot size was 4-m-wide and 6-m-long and planting space was 80 cm × 25 cm. Three replications for each treatment were assigned randomly in the field. Nitrogen fertilizer was applied as urea (46% of N) at 300 kg(N) ha^{-1} of which 40% was applied as a basal dose and 60% at the flower-initiation stage. P [150 kg(P_2O_5) ha^{-1}] and K [150 kg(K_2O) ha^{-1}] were applied as ordinary superphosphate (12% of P_2O_5 and 12% of S) and potassium sulfate (50% of K_2O). Furrow-irrigation was applied in order to minimize the drought stress. Recommended plant protection measures were applied to avoid any biological stress.

Meteorological measurements: The climate data, such as daily temperature (mean daily temperature, mean daily maximum temperature, and mean daily minimum temperature), total precipitation, and sunshine hours were recorded at a climate station (Nanjing weather station) during cotton growing period (Fig. 1*A*). The canopy-level PAR, canopy-level temperature, and relative humidity of each treatment during the flowering and boll-forming stage were shown (Fig. 1*B–D*); it indicated that PAR was a key factor to the shading treatment. In addition, cotton phenology was not essentially changed by shading according to our detailed observation (data not shown). The PAR was measured three times at 0.2 m above the canopy with *AccuPAR LP-80* ceptometer (*Decagon Devices,* Logan, Utah, USA) every 2 h from 06:00 to 18:00 h during a typical clear day. The temperature and relative humidity in the cotton canopy was recorded every 2 h from 06:00 to 18:00 h using hygro-thermometer psychrometer (*DT-8892, CEM,* Shenzhen, China).

Leaf photosynthesis and Chl fluorescence: Cotton white flowers were labeled at anthesis with a tag recording the date at fruiting position 1 and 3 (FP1 and FP3, respectively) of the 7th sympodial branches. Subsequently, the labeled bolls and subtending leaves were measured at 17, 31, and 45 d after anthesis (TAA). The net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s) of the leaves were measured by a portable gas-exchange analyzer (*Li-6400, Li-COR Inc.,* NE, USA) under light intensity of 1,500 μmol(photon) m^{-2} s^{-1}, relative humidity of 65 ± 5%, leaf temperature of 32 ± 2°C, and CO_2 concentration of 380 μmol l^{-1} at 9:30 – 11:00 h.

The leaves were dark-adapted for 30 min using light-exclusion clips before Chl fluorescence measurements using a pulse-amplitude-modulation fluorometer (*PAM 2000, H. WALZ,* Effeltrich, Germany). The minimal fluorescence (F_o) was recorded after the dark for 30 min. The maximal fluorescence (F_m) was measured using saturation pulse [4,000 μmol(photon) m^{-2} s^{-1}] for 0.7 s. The maximal quantum yield was calculated as $(F_m - F_o)/F_m$. The maximal Chl fluorescence in the light-adapted state (F_m’) was measured under 100 μmol(photon) m^{-2} s^{-1} PAR for 15 min and 10 saturation pulses. The effective quantum yield of PSII photochemistry was estimated as $(F_m' - F')/F_m'$ according to Genty *et al.* (1989). Photochemical quenching coefficient (q_P) and nonphotochemical quenching coefficient (q_N) of subtending leaves were calculated

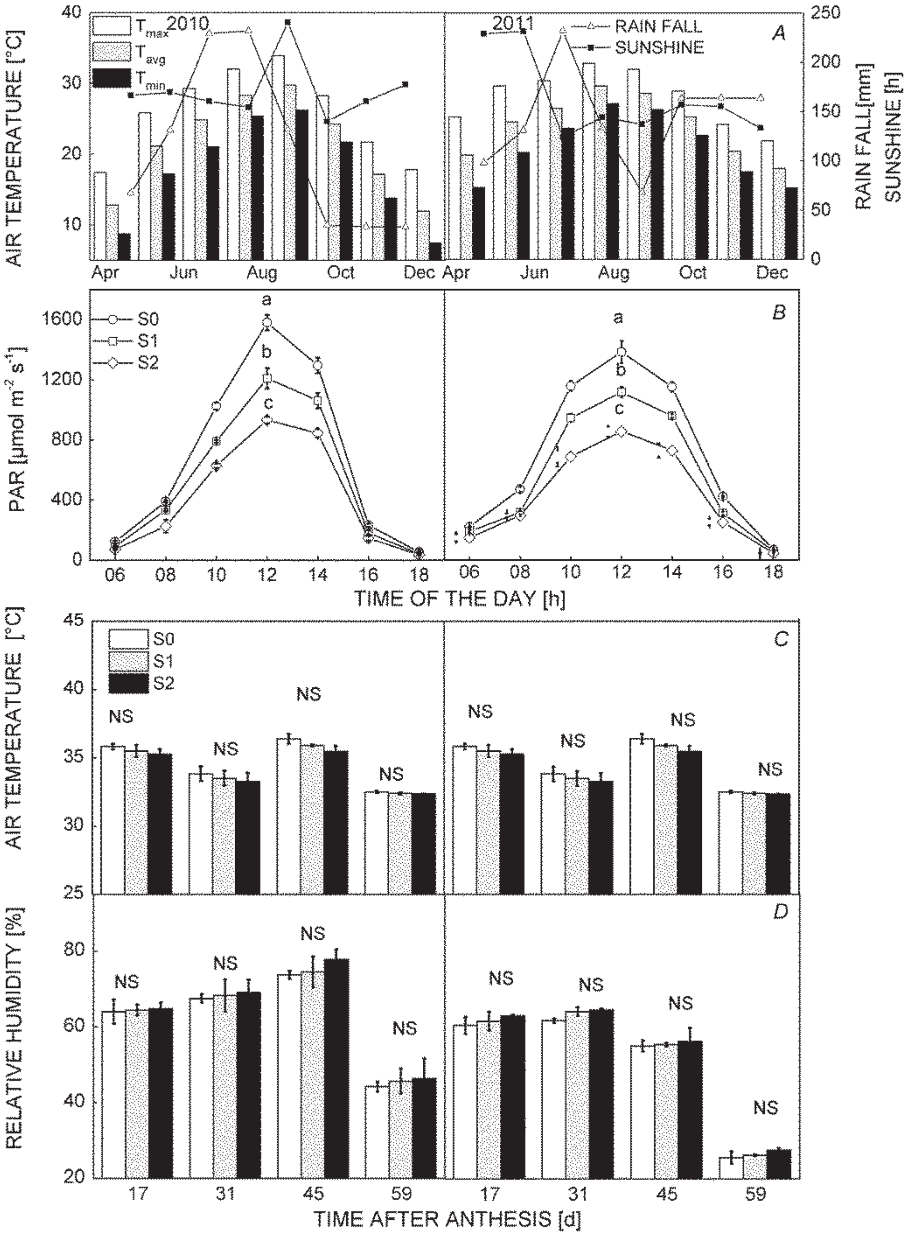

Fig. 1. Climate data during the experimental period. (*A*) Daily minimum, maximum, and mean air temperature, rainfall and sunshine hours of every month during cotton growing period. (*B*) Canopy photosynthetically active radiation (PAR) at 17 days after anthesis of each treatment. S0 – control; S1 and S2 – shading of 21 and 38% of the incident solar radiation, respectively. (*C*) Canopy air temperatures at different growth stages of each treatment. (*D*) Canopy relative humidity at different growth stages of each treatment. Values followed by *the different letters* are significantly different at 0.05 probability level. Each data represents the mean of three replications.

as $(F_m'-F')/(F_m'-F_o')$ and $1-[(F_m'-F_o')/(F_m-F_o)]$. Relative Chl content was measured using a portable chlorophyll meter (*SPAD 502; Minolta Camera,* Osaka, Japan).

Leaf carbohydrate concentrations and leaf mass per area: The leaf samples were collected and brought to the laboratory at 4°C to estimate the fresh mass (FM) and the leaf area (*Li-3000 Area Meter; LI-COR,* Lincoln, NE, USA). Subsequently, the leaves were oven-dried at 105°C for 30 min to determine the dry mass (DM). Leaf mass per area (LMA) was calculated by the ratio between DM and leaf area. Sucrose was extracted and quantified by a modified method of Pettigrew (2001). Briefly, approximately 0.1 g of DM samples were extracted with three successive washes using 5 ml of 80% ethanol. The ethanol samples were incubated at 80°C in a water bath for 30 min. The samples were then centrifuged at 10,000 × *g* for 10 min, and three aliquots of supernatant were collected together for sucrose measurement. The sucrose was measured according to the method described by Hendrix (1993). Leaf samples, which remained from the hot ethanol extraction, were quantified following digestion with pure water boiling for 15 min, then extracted by perchloric acid, and measured with anthrone according to the modified method (Seifter *et al.* 1950).

Cotton yield and fiber properties: The yield was determined each year for both shaded plots and the control. Open bolls were hand-harvested in each plot and total numbers of bolls were calculated by the average of 15 plants in each replication. Lint yield and lint percentage were determined from ginned seed cotton. Five fiber-quality indices were determined at the Cotton Quality Supervision, Inspection and Testing Center of the Ministry of Agriculture in China.

Statistical analysis: Data were statistically analyzed using *SPSS 11.0* (*SPSS Software Inc.,* USA). Treatment means were separated using *Fisher*'s protected least significant difference (LSD) test at 95% level of probability. The changing amplitude (∆%) was calculated as follows: ∆% = [X_{FP1} – X_{FP3}] × 100%/X_{FP1} (Liu *et al.* 2015). The bivariate *Pearson*'s correlations analysis was applied to the obtained data to analyze and confirm the relationships among the lint yield and leaf photosynthesis and Chl fluorescence parameters.

Results

***P*$_N$, *C*$_i$ and *g*$_s$**: Shading caused time-dependent decline of P_N for both fruiting positions (FP1 and FP3) (Fig. 2*A*). At each fruiting position, P_N values under S0 were significantly higher than those under S1 and S2 throughout the growing season, especially at 17 TAA. Furthermore, P_N of subtending leaves at FP3 was lower than that of FP1, which was collected at the same boll age. C_i showed a similar pattern during the growth as P_N (Fig. 2*B*). At 17 TAA, subtending leaves showed the maximum P_N and C_i values. Both FP1 and FP3 under S1 and S2 treatments showed the lower C_i values compared with S0. Similar to P_N, g_s exhibited a significant decrease from S0 to S1 and S2 during the whole period on FP1 and FP3 (Fig. 2*C*). With duration of the boll growth, g_s of subtending leaves tended to decrease, which was similar to C_i. With the change of shading from S0 to S1 or S2, the changing amplitude of P_N and g_s increased, while that of C_i decreased (Table 1).

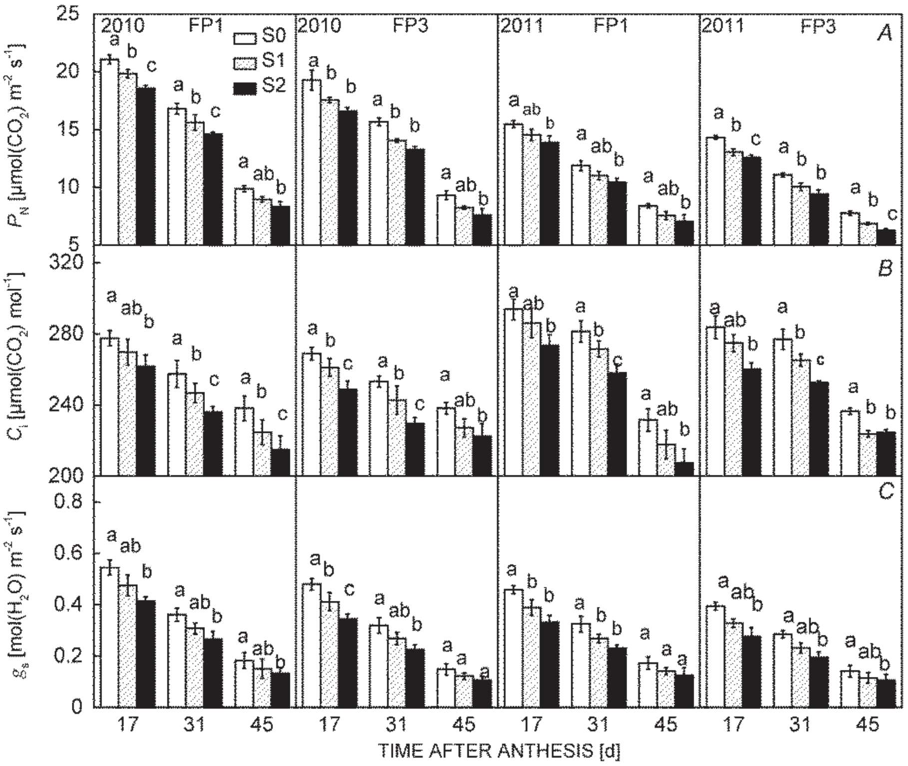

Fig. 2. Effect of shading on P_N, C_i, and g_s values of cotton subtending leaves on FP1 and FP3 during the period of growh in 2010–2011. Values followed by *the different letters* are significantly different at 0.05 probability level. Each data represents the mean of three replications.

Table 1. The changing amplitude of photosynthetic and chlorophyll fluorescence parameters, leaf mass per area, sucrose and starch content of cotton subtending leaves under different shading treatments in 2010–2011. The changing amplitude was calculated as follows: $\Delta\% = [X_{FP1} - X_{FP3}] \times 100\%/X_{FP1}$. TAA − time after anthesis; P_N – net photosynthetic rate; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; Φ_{PSII} – effective quantum yield of PSII photochemistry; F_v/F_m – quantum efficiency of PSII; q_P –quantum efficiency of PSII; q_N – nonphotochemical quenching coefficient, Chl – chlorophyll content, LMA – leaf mass per area; Suc – sucrose content; Sta – starch content.

Year	TAA	Shading treatment	P_N	C_i	g_s	SPAD	Φ_{PSII}	F_v/F_m	q_P	q_N	LMA	Suc	Sta
2010	17	S0	8.5	3.1	12.0	–15.1	8.1	1.2	2.0	–3.6	–8.7	29.0	14.0
		S1	11.6	1.7	13.4	–14.3	8.4	1.2	3.4	–3.7	–8.4	32.5	16.8
		S2	10.6	0.0	16.7	–14.6	8.9	0.6	2.2	–4.2	–7.7	33.5	20.9
	31	S0	6.7	3.2	11.5	–13.9	7.3	1.4	2.3	–3.0	–8.4	29.4	10.7
		S1	10.0	1.6	12.7	–13.2	8.2	0.1	2.6	–3.5	–7.1	31.4	13.0
		S2	9.1	–1.1	15.2	–13.5	8.6	0.2	3.5	–3.2	–7.0	31.0	13.5
	45	S0	5.5	5.0	18.8	–19.5	9.8	1.4	2.3	–1.7	–12.6	33.5	9.5
		S1	7.9	2.8	19.3	–18.5	10.8	1.1	3.0	–1.9	–10.7	29.0	13.7
		S2	9.1	–3.5	19.3	–18.9	10.5	1.2	2.7	–1.8	–11.5	35.8	19.2
2011	17	S0	7.5	3.5	13.9	–16.9	10.1	2.1	2.9	–2.7	–9.2	25.4	19.1
		S1	10.3	1.6	15.6	–16.1	11.0	1.7	3.2	–3.6	–8.4	27.3	22.2
		S2	9.3	–2.1	16.7	–14.3	12.1	1.8	3.6	–4.3	–8.3	30.4	27.6
	31	S0	6.7	3.9	12.4	–20.6	9.6	2.0	2.0	–1.7	–10.9	26.1	13.9
		S1	8.7	2.3	13.8	–20.1	11.5	0.7	3.5	–2.0	–9.2	29.1	16.4
		S2	9.8	–2.8	15.9	–19.3	10.9	0.0	4.5	–2.7	–10.2	33.2	15.1
	45	S0	7.7	4.9	17.9	–25.9	7.8	2.2	2.8	–1.1	–18.7	12.0	3.1
		S1	9.2	2.1	18.6	–24.5	8.5	1.8	3.7	–1.2	–16.4	12.6	8.5
		S2	11.2	–8.2	16.4	–24.7	9.6	1.2	3.7	–1.7	–17.5	14.0	9.7

Chl content and Chl fluorescence: The SPAD values for the shading treatments were not significantly different at both FP1 and FP3 (Fig. 3*A*). There was a decrease in effective quantum yield of PSII photochemistry (Φ_{PSII}) during the boll-forming stage (Fig. 3*B*), with the maximum Φ_{PSII} value occurring at 17 TAA in S0 on FP1. Φ_{PSII} values showed a decreasing trend when the shading was changed from S0 to S2 at both FP1 and FP3. In our study, the ratio of F_v/F_m varied depending on the growth stage and the shading treatment at each fruiting position (Fig. 3*C*). The ratio of F_v/F_m in FP1 and FP3 increased by both S1 and S2. There were fluctuations both in q_P and q_N values during the growth period and both q_P and q_N were significantly affected by S1 or S2 (Fig. 3*D*,*E*). Shading decreased q_P, while increased q_N at both FP1 and FP3 at every stage, indicating that low light intensity diminished primary stable quinine acceptor of PSII (Q_A), reduced the activity of electron transport through PSII, and enhanced the thermal dissipation. The changing amplitude of Φ_{PSII}, F_v/F_m, and q_P were positive, while those of SPAD and q_N were negative, indicating that the photochemical efficiency on FP3 were lower than that on FP1 (Table 1). Shading increased the absolute values of changing amplitude of Φ_{PSII}, q_P, and q_N, indicating that low light intensity enlarged the differences of these three photochemical parameters between FP1 and FP3.

Leaf mass per area and carbohydrate content: It was clear that photosynthesis was correlated to leaf mass per area (LMA) based on measurement at both single leaf and canopy levels. Similar to the behavior of P_N, LMA of the leaves under shading was significantly reduced compared with the control leaves at all growth stages. However, LMA increased with TAA on both FP1 and FP3, which was opposite to a trend of P_N (Fig. 4*A*). Meanwhile, the changing amplitude of LMA were highly negative, indicating that leaves on FP3 gained much higher leaf mass per area than that of FP1. Although shading decreased LMA at every boll growing stage, the absolute changing amplitude of LMA decreased, indicating that shading reduced differences between FP1 and FP3 (Table 1).

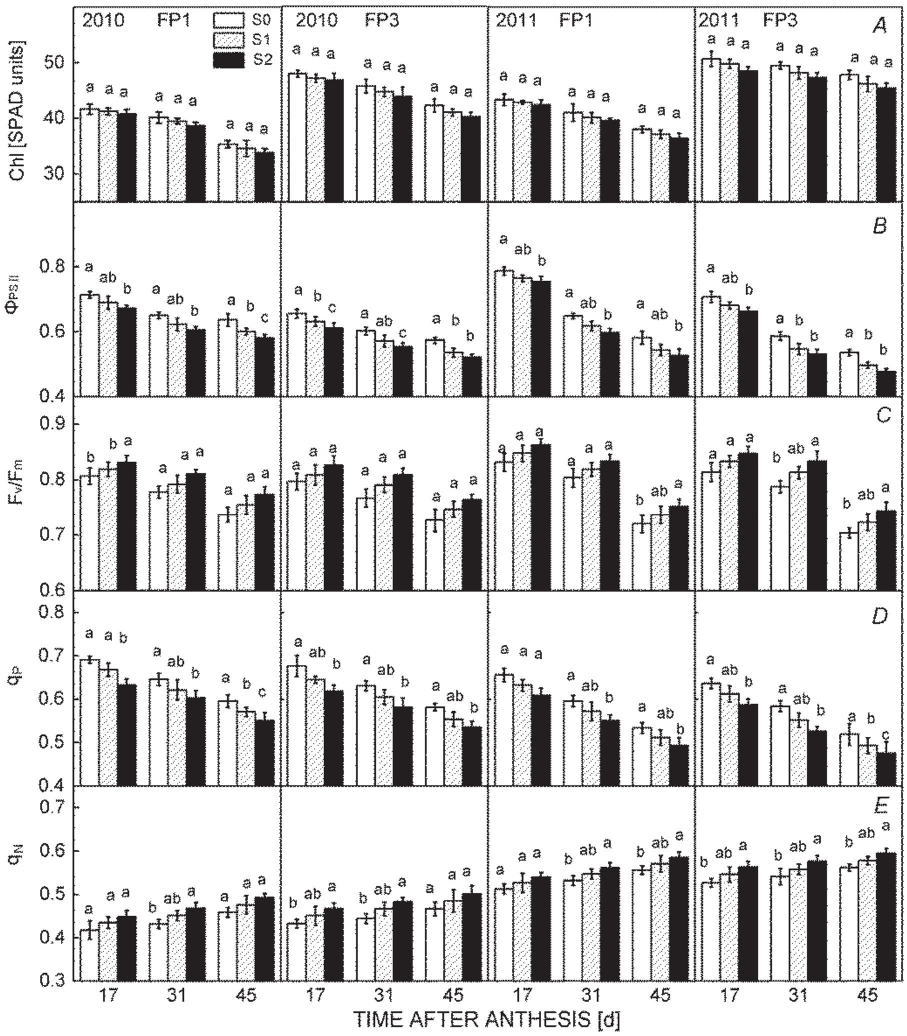

Fig. 3. Effect of shading on Chl content, Φ_{PSII}, F_v/F_m, q_P, and q_N values of cotton subtending leaves on FP1 and FP3 with time course in 2010–2011. Values followed by *the different letters* are significantly different at 0.05 probability level. Each data represents the mean of three replications.

The sucrose and starch contents in the subtending leaves under S1 and S2 were lower than those under S0 (Fig. 4*B,C*). Sucrose contents were reduced by approximately 9.4% (in S1) and 19.7% (in S2) on FP1, and by 10.7% (in S1) and 23.8% (in S2) on FP3 compared with those in the control leaves. Leaves from the most heavy shaded plants (S2) exhibited 42.1, 26.5, and 33.6% on FP1, and 47.5, 28.2, and 39.5% on FP3 lower starch contents compared with the control leaves at 17, 31, and 45 TAA, respectively. The changing amplitudes of sucrose and starch contents were highly positive, and shading increased both of the values (Table 1), indicating that both sucrose and starch contents were higher on FP1 than that of FP3, and shading enlarged the variation of a fruiting-position effect.

Cotton yield and fiber quality: Table 2 shows the lint yield, yield components, and fiber properties of different shading treatments. Boll numbers, boll mass, and the lint yield significantly decreased under shading treatments compared with those of S0. Shading also significantly increased fiber length and decreased fiber strength and micronaire in 2010 and 2011 (Table 2). The cotton yield in 2011 was higher than that in 2010 (Table 2). The differences in the lint yield and boll numbers between the two years were significant, indicating that the response of boll forming to climate varied with boll retention rate. Thus, we compared the changing amplitude at different fruiting positions with the shading treatment. Shading increased the changing amplitude of boll numbers, boll mass, lint yield, micronaire, and fiber strength, while decreased that of fiber length (Table 3), indicating that shadings aggravated the changing amplitude of fruiting position on cotton yield components (boll numbers and boll mass) and fiber properties (fiber strength and micronaire).

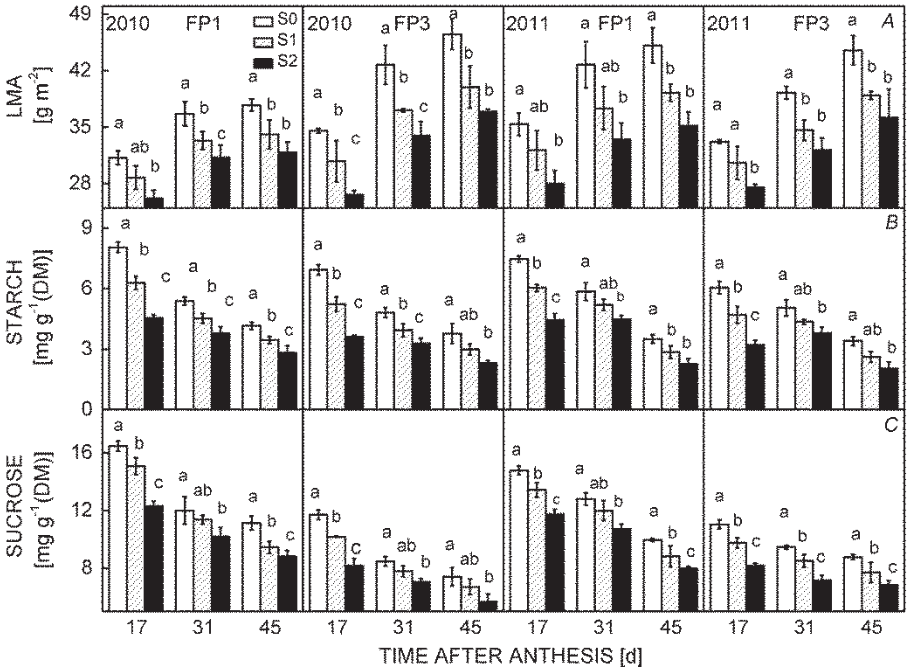

Fig. 4. Effect of shading on leaf mass per area, sucrose and starch contents of cotton subtending leaves on FP1 and FP3 with time in 2010–2011. Values followed by *the different letters* are significantly different at 0.05 probability level. Each data represents the mean of three replications.

Table 2. Effect of shading on lint yield, yield component, and fiber properties of cotton in 2010 – 2011. S0 – control; S1 and S2 – shading of 21 and 38% of the incident solar radiation; respectively. BN, BW, LP, LY, UHM, ST, and MIC – boll numbers, boll mass, lint percentage, lint yield, fiber length, fiber strength, and fiber micronaire, respectively. Each data represents the mean of three replications. Values followed by *the different letters* within the same column are significantly different at 0.05 probability level. * and ** – significant at 5% and 1% probability level, respectively.

Year	Shading treatment	BN [number m^{-2}]	BW [g boll^{-1}]	LP [%]	LY [kg ha^{-1}]	UHM [mm]	ST [cN tex^{-1}]	MIC
2010	S0	86.5^{a}	5.2^{a}	37.7^{a}	1703^{a}	30.0^{a}	29.0^{a}	4.4^{a}
	S1	78.5^{b}	4.7^{b}	37.0^{b}	1374^{b}	29.8^{b}	28.5^{b}	4.2^{a}
	S2	71.5^{c}	4.4^{c}	36.3^{c}	1145^{c}	29.6^{b}	27.7^{c}	3.9^{b}
	LSD$_{0.05}$	**	**	**	**	**	**	**
2011	S0	101.0^{a}	5.3^{a}	36.3^{a}	1930^{a}	29.6^{a}	28.6^{a}	4.4^{a}
	S1	90.5^{b}	5.0^{b}	35.4^{b}	1611^{b}	29.3^{b}	28.0^{b}	4.4^{a}
	S2	84.5^{c}	4.7^{c}	34.0^{c}	1346^{c}	29.3^{b}	27.5^{c}	4.2^{b}
	LSD$_{0.05}$	**	**	**	**	*	**	*

Table 3. The changing amplitude of lint yield, yield component, and fiber properties of cotton under different shading treatments in 2010–2011. BN, BM, LP, LY, UHM, ST, and MIC – boll numbers, boll mass, lint percentage, lint yield, fiber length, fiber strength, and fiber micronaire, respectively.

Year	Shading treatment	BN	BM	LP	LY	UHM	ST	MIC
2010	S0	59.1	5.4	1.3	61.8	3.8	4.2	7.0
	S1	62.5	6.8	1.1	65.1	3.3	4.7	10.7
	S2	65.0	6.7	1.0	67.8	2.8	5.0	14.2
2011	S0	59.7	5.5	0.7	62.0	4.0	5.9	8.6
	S1	63.6	6.0	0.5	65.9	3.1	6.2	10.2
	S2	66.2	6.7	0.6	68.7	3.0	6.7	11.3

Table 4. Correlations among the changing amplitude of different shading treatments (S) on photosynthetic and Chl fluorescence parameters and lint yield and physiological characteristics. S_{PN}, S_{Ci}, S_{gs}, $S_{\Phi PSII}$, $S_{Fv/Fm}$, S_{qP}, S_{qN}, S_{Chl}, S_{LMA} and S_{LY} represent the changing amplitude of different shadings on net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), effective quantum yield of PSII photochemistry (Φ_{PSII}), quantum efficiency of PSII (F_v/F_m), photochemical quenching coefficient (q_P), nonphotochemical quenching coefficient (q_N), chlorophyll content (Chl), leaf mass per area (LMA), and lint yield (LY), respectively. The changing amplitude was calculated as follows: $\Delta\% = [X_{FP1} - X_{FP3}] \times 100\%/X_{FP1}$. Values of the variation of photosynthetic and Chl fluorescence parameters, LMA and Chl content were mean of all the stages. $n = 8$, $R^2_{0.05} = 0.707$ $R^2_{0.01} = 0.834$, * and ** – significant effects at 0.05 levels and 0.01 levels, respectively. Minus indicates negative correlation between two variables.

Correlation to	S_{PN}	S_{Ci}	S_{gs}	S_{Chl}	$S_{\Phi PSII}$	$S_{Fv/Fm}$	S_{qP}	S_{qN}	S_{LMA}	S_{LY}
S_{PN}	1									
S_{Ci}	0.982**	1								
S_{gs}	0.939**	0.979**	1							
S_{Chl}	0.955**	0.970**	0.929**	1						
$S_{\Phi PSII}$	0.967**	0.987**	0.956**	0.994**	1					
$S_{Fv/Fm}$	–0.983**	–0.995**	–0.968**	–0.985**	–0.995**	1				
S_{qP}	0.971**	0.990**	0.986**	0.964**	0.978**	–0.990**	1			
S_{qN}	–0.887**	–0.837**	–0.829*	–0.768*	–0.795*	0.846**	–0.870**	1		
S_{LMA}	0.951**	0.940**	0.943**	0.868**	0.898**	–0.934**	0.960**	–0.961**	1	
S_{LY}	0.930**	0.920**	0.833*	0.948**	0.946**	–0.934**	0.875**	–0.705	0.785*	1

The correlations among the variation of shading treatment on lint yield and photosynthetic and fluorescence parameters: It is well documented that leaves with higher photosynthesis provided more assimilates to developing bolls. Significant correlations were found among the variation of different shading treatments on photosynthetic and Chl fluorescence parameters and lint yield and physiological characteristics (Table 4). Moreover, it was found that the changing amplitude of P_N, C_i, g_s, Φ_{PSII}, q_P, leaf mass per area, and lint yield were positively correlated with shading, while the changing amplitude of F_v/F_m and q_N were negatively correlated with shading. These results indicated that low irradiance environment (shading treatment) relative to the control produced the lower lint yield, which was mainly associated with the variation of photosynthesis of the subtending leaves on both FP1 and FP3.

Discussion

Under the conditions of our study, P_N and g_s of both FP1 and FP3 were strongly correlated to the shading (Fig. 2), with boll mass and boll numbers being increasingly compromised as the level of shading increased (Table 2). Zhao and Oosterhuis (1994) also reported that low light intensity decreased the photosynthetic rate of main-stem cotton leaves. Many studies indicated that low irradiance limits the photosynthesis and leaf gas exchange, mainly in young leaves (Medina *et al.* 2002, Yoshimura 2010, Mauro *et al.* 2011, Echer and Rosolem 2015a). The stomatal behavior is subject to the feedback of photosynthesis activity and stomatal opening is one of the major limitations for photosynthesis (Cornic 1994). In general, larger stomatal opening and conductance are favorable for CO_2 entrance into mesophyll cell, and as a consequence, photosynthesis enhances. In our study, shading significantly decreased g_s and C_i, which could be due to the ability of the mesophyll cell to fix carbon and the observed decrease in C_i itself was an indirect additional effect of shading. The significant decrease in C_i of subtending leaves appeared at every stage of FP1 and FP3, which were collected at same leaf age. Results indicated that the mesophyll cell were more sensitive to shading on FP3 than that of FP1.

On the other hand, a decreased PSII photochemical efficiency could also be attributed to P_N decline (Chen *et al.* 2005). Here, we showed that the value of Φ_{PSII} and q_P were reduced by shading at both FP1 and FP3 (Fig. 3), indicating an alteration in the photochemical efficiency. Moreover, shading caused the reduction in the relative Chl content, as inferred from SPAD readings, and increased F_v/F_m (Fig. 3), which was indicative of a strong increase in PSII photochemical efficiency (Mauro *et al.* 2011, Pettigrew 1995). The results indicated that shanding not only decreased the ligh interception but also decreased the photochemical reaction to maintan a high levels of heat dissipation (Mu *et al.* 2008). Presumably, shading affects PSII reaction centers, diminishes the stable quinine acceptor of PSII (Q_A), decreases the activity of electron transport through PSII, and causes the decrement of q_P. At the same time, light energy adsorbed by antenna pigment under shading condition cannot be used for the electron transport, but it is dissipated as thermal energy, leading to an increment of q_N. These results were consistent with and supported by results obtained from *Capsicum anmuum* L (Zhu *et al.* 2012). The highest changing amplitude of Φ_{PSII} indicated that fruiting positions affected Chl fluorescence mainly through the quantum efficiency of electron transport through PSII.

LMA of the leaves under the shading treatments was reduced compared with the control leaves at both FP1 and FP3 in our study (Fig. 4), which is consistent with the results of Pettigrew (2001). It is well documented that a strong relationship exists between canopy photosynthesis and LMA (Thompson *et al.* 1995, Bondada and Oosterhuis 2001), and reduced LMA could reflect the decrease of photosynthetic capacity and the activity of whole plant population, which ultimately leads to the decline of the yield. However, some researchers believed that leaf photosynthesis alone does not demonstrate a close association with the yield, and the LMA should be used with caution to index P_N (Bondada and Oosterhuis 2001). It has been suggested that the primary source of photosynthate (mainly nonstructural carbohydrates) for a boll is its subtending leaf (Ashley 1972), which was supported by our results. S_{P_N} was highly correlated with S_{LY} (R^2 =0.930**), and S_{P_N} was highly correlated with S_{LMA} (R^2 = 0.951**). The correlations among the changing amplitude of different shading treatments on photosynthetic and Chl fluorescence parameters and lint yield were all significantly high (Table 4). Presumably, the reduced yield (mainly on boll mass and boll number) under the shade was caused by lower photosynthetic rates and photochemical efficiency because of the lower light intensity.

Previous studies have suggested that the variation of starch and sucrose in cotton fibers is associated with the fiber quality (Pettigrew 2001). The photosynthates partitioned to the developing boll (to either the ovule or the fiber) were primary from the subtending leaves of the boll (Ryser 1992) and the content of sucrose and starch in cotton subtending leaves was higher than hexose (Zhao and Oosterhuis 1998a, Pettigrew 2001). Therefore, it is pertinent to examine the content and variation of sucrose and starch that might affect a fiber quality. Shading significantly decreased sucrose and starch contents of boll's subtending leaves at both FP1 and FP3, which was consistent with the results of Zhao and Oosterhuis (1998a) and Pettigrew (2001). The changing amplitude of sucrose and starch contents was highly positive, and increased by shading, which indicated that differences between FP1 and FP3 were significant and shading could enlarge these differences. Therefore, under low light intensity or on distal positions of fruiting branches, boll abscission rate was higher and the fiber quality decreased.

Leaf photosynthesis and photoassimilate allocation are essential for crop growth and the yield (Zhao *et al.* 2004). The leaf adjacent to the boll is apparently the primary source of photosynthates for boll growth (Ashley 1972, Constable and Rawson 1980, Wullschleger and Oosterhuis 1990). Fiber strength and micronaire, which were associated with the cellulose deposition within fibers and the developmental process (Wang *et al.* 2009). Environmental conditions altering the relationship between the photosynthetic assimilate source and the reproductive sink would affect fiber strength and micronaire formation (Lv *et al.* 2004). However, the alternation in the assimilate supply available to the developing bolls affects fiber quality less directly than the yield (Pettigrew 2001). Therefore, better understanding of the responses of single-leaf photosynthesis was much important in order to enhance the crop yield and improve the fiber quality (Peng 2000, Sun *et al.* 2009).

Previous studies demonstrated that in cotton under simulated shading conditions bolls make an adaptive adjustment to light-limited environment, such as abortion probability and potential sink size (Dusserre *et al.* 2002). In the present study, the lint yield significantly decreased at both FP1 and FP3 in 2010 and 2011 (Table 2). Shading had a significant effect on boll mass and boll number, but not on lint percentage, which is partially consistent with the previous results (Pettigrew 1994, Zhao and Oosterhuis 1998b). Shading enlarged the differences between FP1 and FP3 in boll numbers and lint yield (Table 2). The adaxial position showed a higher boll retention rate than the distal position (Heitholt 1993, Jenkins *et al.* 1990, Anjum *et al.* 2001, Boquet and Moser 2003), which is due to the fact that the adaxial position exhibited a higher photosynthetic capacity than that of the distal position. The higher cotton yield over the year was attributed to a higher boll numbers (Table 2) due to a better climate at the flowering and boll-forming stage. Fiber quality is mainly associated with nutritional and environmental conditions during the boll development. The results reported here revealed that fiber length, strength, and micronaire values on both FP1 and FP3 were significantly sensitive to low light intensity during the boll development (Table 2), which is consistent with previous observation that long-term shading decreased the strength and micronaire of cotton fiber (Pettigrew 1995). The effect of shading on the fiber length differs in different studies. Wang *et al.* (2005) considered that shading decreased the fiber length, while a large proportion of studies believed that fiber length was not responsive to the shading treatment during the boll development (Zhao and Oosterhuis 2000a, Echer and Rosolem 2015b). Our results were consistent with Pettigrew (1995) showing that shadings decreased the fiber length. Unaffected fiber length observed by Zhao *et al.* (2000a) and Echer (2015b) was due to different percentage of shading. The most sensitive parameter to low light was fiber micronaire followed by fiber strength and fiber length (Table 3). The fiber at the adaxial position showed the higher fiber length than that of the distal position (Heitholt and Schmidt 1994, Heitholt 1997, Pettigrew 2001, Davidonis *et al.* 2004). Fiber strength and micronaire values at FP1 were also higher than that at FP3 in our study, and similar results were also observed by Heitholt *et al.* (1994) and Pettigrew (1995) in their source-to-sink manipulation effects on cotton fiber quality among different fruiting positions.

Conclusion: This study provided information on effects of simulated shading conditions on photosynthesis, carbohydrate concentrations in subtending leaves of cotton boll, as well as cotton fiber yield and quality. Compared to photosynthetic parameters, there were relatively small position effects on four fluorescence parameters under shading treatments. However, photosynthetic and fluorescence parameters were significantly correlated with the shading treatments. Sucrose and starch contents of subtending leaves were also significantly reduced by the shading on both FP1 and FP3. Lint yield and fiber quality were significantly reduced under the light-limited environment, and the effect impacted more bolls at FP3 than that of FP1. Our results demonstrated that the changes of photosynthesis on subtending leaves greatly contributed to the final yield and fiber properties under shading treatments at both FP1 and FP3. Farmers can reduce the yield and quality loss by sowing crop at optimal time in order to evade shading at the flowering and boll formation stage. Moreover, reduced row to row distance tends to decrease the appearance of FP3, since it could be a strategy to mitigate the effect of shading on boll retention.

References

Anjum R., Soomro A., Chang M. *et al.*: Effect of fruiting positions on yield in American cotton. – Pak. J. Biol Sci. **4**: 960-962, 2001.

Ashley D.A.: C-labelled photosynthate translocation and utilization in cotton plants. – Crop Sci. **12**: 69-74, 1972.

Bondada B.R., Oosterhuis D.M.: Canopy photosynthesis, specific leaf weight, and yield components of cotton under varying nitrogen supply. – J. Plant Nutr. **24**: 469-477, 2001.

Boquet D.J., Moser E.B.: Boll retention and boll size among intrasympodial fruiting sites in cotton. – Crop Sci. **43**: 195-201, 2003.

Chen Z., Wu F., Wang X*, et al.*: Heterosis in CMS hybrids of cotton for photosynthetic and chlorophyll fluorescence parameters. – Euphytica **144**: 353-361, 2005.

Chen J., Lv F., Liu J. *et al.*: Effects of different planting dates and low light on cotton fibre length formation. – Acta Physiol Plant. **36**: 2581-2595, 2014.

Constable G.A., Rawson H.M.: Carbon production and utilization in cotton: inferences from a carbon budget. – Aust. J. Plant Physiol. **7**: 539-553, 1980.

Cornić G.: Drought stress and high light effects on leaf photosynthesis. – In: Baker N.R., Bowyer J.R. (ed.): Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field. Pp. 297-313. Bios Scientific Publishers, Oxford 1994.

Davidonis G.H., Johnson A.S., Landivar J.A. *et al.*: Cotton fiber quality is related to boll location and planting date. – Agron. J. **96**: 42-47, 2004.

Dusserre J., Crozat Y., Warembourg F.R. *et al.*: Effects of shading on sink capacity and yield components of cotton in controlled environments. – Agronomie **22**: 307-320, 2002.

Echer F.R., Rosolem C.A.: Cotton leaf gas exchange responses to irradiance and leaf aging. – Biol. Plantarum **59**: 366-372, 2015a.

Echer F.R., Rosolem C.A.: Cotton yield and fiber quality affected by row spacing and shading at different growth stages. – Eur. J. Agron. **65**: 18-26, 2015b.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Gu L.L., Wang X.S., Zhou Z.G. *et al.*: [Researches of high yield cotton cultivations in Jiangsu province.] – China Cotton **4**: 14-16, 2010. [In Chinese]

Heitholt J.J.: Floral bud removal from specific fruiting positions in cotton: Yield and fiber quality. – Crop Sci. **37**: 826-832, 1997.

Heitholt J.J.: Cotton boll retention and its relationship to lint yield. – Crop Sci. **33**: 486-490, 1993.

Heitholt J.J., Schmidt J.H.: Receptacle and ovary assimilate concentrations and subsequent boll retention in cotton. – Crop Sci. **34**: 125-131, 1994.

Hendrix D.L.: Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. – Crop Sci. **33**: 1306-1311, 1993.

Jenkins J.N., McCarty J., Parrott W.: Fruiting efficiency in cotton: Boll size and boll set percentage. – Crop Sci. **30**: 857-860, 1990.

Kerby T., Buxton D.: Competition between adjacent fruiting forms in cotton. – Agron. J. **73**: 867-871, 1981.

Liu J.R., Meng Y.l., Chen J. *et al.*: Effect of late planting and shading on cotton yield and fiber quality formation. – Field Crop. Res. **183**:1-13, 2015.

Lv F.J., Liu J.R., Ma Y.N. *et al.*: Effect of shading on cotton yield and quality on different fruiting branches. – Crop Sci. **53**: 2670-2678, 2013.

Mauro R., Occhipinti A., Longo A. *et al.*: Effects of shading on chlorophyll content, chlorophyll fluorescence and photosynthesis of subterranean clover. – J. Agron. Crop Sci. **197**: 57-66, 2011.

Medina C.L., Souza R.P., Machado E.C. *et al.*: Photosynthetic response of citrus grown under reflective aluminized polypropylene shading nets. – Sci. Hortic.-Amsterdam **96**: 115-125, 2002.

Mu H., Jiang D., Dai T.B. *et al.*: Effect of shading on photosynthesis and chlorophyll fluorescence characters in wheat flag leaves. – Agric. Sci. China **41**: 599-606, 2008.

Oosterhuis D.M.: Growth and development of a cotton plant. – In: Miley W.N., Oosterhuis D.M. (ed.): Nitrogen Nutrition of Cotton: Practical Issues. Pp. 1-24. ASA, Madison 1990.

Peng S.B.: Single-leaf and canopy photosynthesis of rice. – Stud. Plan. S. **7**: 213-228, 2000.

Pettigrew W.T.: Environmental effects on cotton fiber carbohydrate concentration and quality. – Crop Sci. **41**: 1108-1113, 2001.

Pettigrew W.T.: Cotton genotypic variation in the photosynthetic response to irradiance. – Photosynthetica **42**: 567-571, 2004.

Pettigrew W.T, Meredith W.R.: Genotypic variation in physiological strategies for attaining cotton lint yield production. – J. Cotton Sci. **16**: 179-189, 2012.

Pettigrew W.T., Meredith W.R.: Leaf gas exchange parameters vary among cotton genotypes. – Crop Sci. **34**: 700-705, 1994.

Pettigrew W.T., Turley R.: Variation in photosynthetic components among photosynthetically diverse cotton genotypes. – Photosynth. Res. **56**: 15-25, 1998.

Pettigrew W.T.: Source-to-sink manipulation effects on cotton lint yield and yield components. – Agron. J. **86**: 731-735, 1994.

Pettigrew W.T.: Source-to-sink manipulation effects on cotton fiber quality. – Agron. J. **87**: 947-952, 1995.

Ryser U.: Ultrastructure of the epidermis of developing cotton (*Gossypium*) seeds: suberin, pits, plasmodesmata, and their implication for assimilate transport into cotton fibers. – Am. J. Bot. **79**: 14-22, 1992.

Seifter S., Dayton S., Novic B. *et al.*: The estimation of glycogen with the anthrone reagent. – Arch. Biochem. **25**: 191-200, 1950.

Sun C., Qi H., Hao J. *et al.*: Single leaves photosynthetic characteristics of two insect-resistant transgenic cotton (*Gossypium hirsutum* L.) varieties in response to light. – Photosynthetica **47**: 399-408, 2009.

Thompson J.A., Nelson R.L., Schweitzer L.E.: Relationships among specific leaf weight, photosynthetic rate, and seed yield in soybean. – Crop Sci. **35**: 1575-1581, 1995.

Wang Q.C., Wang Z.L., Song X.L. *et al.*: [Effects of shading at blossoming and boll-forming stages on cotton fiber quality.] – Chin. J. Appl. Ecol. **16**: 1465-1468, 2005. [In Chinese]

Wang Y., Shu H., Chen B. *et al.*: The rate of cellulose increase is highly related to cotton fibre strength and is significantly determined by its genetic background and boll period temperature. – Plant Growth Regul. **57**: 203-209, 2009.

Wullschleger S.D., Oosterhuis D.M.: Photosynthetic carbon production and use by developing cotton leaves and bolls. – Crop Sci. **30**: 1259-1264, 1990.

Yoshimura K.: Irradiance heterogeneity within crown affects photosynthetic capacity and nitrogen distribution of leaves in *Cedrela sinensis*. – Plant Cell. Environ. **33**: 750-758, 2010.

Zeng B., Xu X., Zhou S. *et al.*: Effects of temperature and light on photosynthetic heterosis of an upland cotton hybrid cultivar. – Crop Sci. **52**: 282-291, 2012.

Zhang L., Zhang G., Wang Y. *et al.*: Effect of soil salinity on physiological characteristics of functional leaves of cotton plants. – J. Plant Res. **126**: 293-304, 2013.

Zhao D., Oosterhuis D.M.: Effects of shading on cotton photosynthesis, yield and yield components. – In: Proceedings of the 2000 Cotton Research Meeting and Summaries of Cotton Research in Progress, Arkansas Agriculture Experiment Station Special report. **198**: 131-135, 1994.

Zhao D., Oosterhuis D.M.: Cotton responses to shade at different growth stages: Nonstructural carbohydrate composition. – Crop Sci. **38**: 1196-1203, 1998a.

Zhao D., Oosterhuis D.M.: Physiologic and yield responses of shaded cotton to the plant growth regulator PGR-IV. – J. Plant Growth Regul. **17**: 47-52, 1998b.

Zhao D., Oosterhuis D.M.: Cotton responses to shade at different growth stages: growth, lint yield and fibre quality. – Exp. Agr. **36**: 27-39, 2000a.

Zhao D., Oosterhuis D.M.: Nitrogen application effect on leaf photosynthesis, nonstructural carbohydrate concentrations and yield of field-grown cotton. – In: Oosterhuis D.M. (ed.): Proceedings of the 2000 Arkansas Cotton Research, AAES Special Report. **198**: 69-71, 2000b.

Zhao D., Reddy K.R., Kakani V.G. *et al.*: Leaf and canopy photosynthetic characteristics of cotton (*Gossypium hirsutum*) under elevated CO_2 concentration and UV-B radiation. – J. Plant Physiol. **161**: 581-590, 2004.

Zhu J.J., Qiang P., Liang Y.L. *et al.*: Leaf gas exchange, chlorophyll fluorescence, and fruit yield in hot pepper (*Capsicum anmuum* L.) grown under different shade and soil moisture during the fruit growth stage. – J. Integr. Agr. **11**: 927-937, 2012.