BIOLOGIA PLANTARUM 59 (2): 366-372, 2015 DOI: 10.1007/s10535-015-0484-3

Cotton leaf gas exchange responses to irradiance and leaf aging

F.R. ECHER[1]* and C.A. ROSOLEM[2]

Mato Grosso Cotton Institute, Primavera do Leste, MT, 78850-00 Brazil[1]
College of Agricultural Sciences, São Paulo State University, Botucatu, SP, 18610-307 Brazil[2]

Abstract

Cotton (*Gossypium hirsutum* L.) net photosynthetic rate (P_N) decreased with leaf age and low irradiance, and the interaction of these factors might lead to decreased yields mainly in crops with a high plant density. Shade decreased leaf gas exchange, mainly in young leaves, which did not recover after exposure to an increasing irradiance. Old leaves reached P_N saturation at a lower irradiance than young leaves. Also stomatal conductance decreased with leaf age and shading. Intercellular CO_2 concentration and irradiance were inversely related. The responses of P_N to irradiance decreased as leaf aged, and it did not interact with shade suggesting that self-shading is not primarily responsible for the decreased photosynthetic rate in older cotton leaves.

Additional key-words: *Gossypium hirsutum,* intercellular CO_2 concentration, P_N, shading, stomatal conductance.

Introduction

The activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is regulated by irradiance (Taiz and Zeiger 2010) and hence the net photosynthetic rate (P_N) and sugar content in cotton leaves and bolls decrease under low irradiance (Zhao and Oosterhuis 1998a). On cloudy days, frequent in cotton growing regions in the tropics, sugar depletion results in abscission of reproductive structures and eventually in yield loss (Wells 2011). In addition to shading, leaf aging decreases the photosynthetic capacity, *e.g.*, due to a modification in the number and size of chloroplasts (Pettigrew 2004). Furthermore, the decrease in P_N with leaf aging is significantly correlated with a decrease in free indole-3 acetic acid (Guinn and Brummett 1993), the content of chlorophyll and soluble proteins, and Rubisco activity (Wells 1988). In addition, nitrogen is remobilized to younger tissues and to fruits at the bottom of the canopy as the crop ages. Milroy *et al*. (2001) found a positive relationship between N content per leaf area unit in each canopy layer and relative irradiance.

The planophile structure of cotton leaves results in a strong gradient of irradiance through the canopy. The photosynthetically active radiation (PAR) in the upper cotton leaves is close to 100 % of incident PAR. However, values as low as 20, 10, and 5 % of incident PAR are observed in the plant bottom at plant density of 1, 2, and 4 plants m^{-2} (Dauzat *et al*. 2008), respectively. Leaves grown under low irradiance exhibit a saturated P_N at a lower irradiance than leaves developed under a high irradiance (Landivar *et al*. 2010). This is particularly important in cropping systems with a high plant density because the penetration of radiation through the crop canopy is related to the leaf area. Leaf size, arrangement, angle, and crop density/row space affect the interaction of these factors (Saeki 1963). Brodrick *et al*. (2013) reported an increased coefficient of absorbance from 0.69 with 12 plants m^{-2} to 0.81 with 36 plants m^{-2}. Even so, a higher absorbance does not increase a total biomass yield because the radiation use efficiency is low. Most studies on P_N in cotton were developed after leaves were acclimated to various irradiances. However, a different response might be forthcoming under rapid changes in irradiance as it occurs with partly cloudy sky conditions (Bauer *et al.* 1997).

In tropical areas with long growing seasons, cotton sowing dates have been facing changes, so as to grow two crops in the same season: early soybeans followed by cotton. Hence, cotton has been sown late in the season at

---

Submitted 15 October 2013, *last revision* 2 July 2014, *accepted* 8 July 2014.

Abbreviations: c_a - atmospheric CO_2 concentration; c_i - intercellular CO_2 concentration; g_s - stomatal conductance; PAR - photosynthetically active radiation; P_N - net photosynthetic rate; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase.

Acknowledgments: We acknowledge CAPES (the Brazilian Federal Agency for the Improvement of Higher Education) for granting a PhD scholarship to the first author.

* Corresponding author; fax: (+55) 6634982267, e-mail: fabioecher@gmail.com

increased plant populations, or narrow rows, to compensate for a lower yield per plant. When cotton is cropped late in the season, bolls develop under shorter days and, eventually, less radiation energy is available. Self-shading under a high density may further impair P_N and decrease yield. If the amount of absorbed radiation is not high enough, the effect of leaf aging may be less important in decreasing P_N than the effect of irradiance. The knowledge of shading effects on the cotton physiological characteristics can help understand plant behavior in cropping systems with a high plant density. To prove a hypothesis that a decrease in P_N in cotton leaves by shading could be more important than by leaf aging, this study aimed at evaluating the effects of leaf age and irradiance, alone or in combination, on cotton leaf gas exchange.

Materials and methods

Three experiments were carried out in a greenhouse where cotton (*Gossypium hirsutum* L. var. *latifolia*, cv. FMT 701) was grown in pots filled with soil. The pots were irrigated daily with distilled water, and a half-strength Hoagland nutrient solution was applied weekly. The average greenhouse CO_2 concentration was 388 ± 6 µmol mol^{-1}, day/night temperatures of 34/20°C, and relative humidities of 50/90%. Daily PAR values and a day length are shown in Fig. 1. Experiment 1 was conducted from 17 November 2009 to 30 January 2010; experiment 2 from 1 to 30 February 2010, and experiment 3 from 2 February to 25 April 2010. A supplemental radiation was not used. In shaded treatments, the irradiance was decreased to 50 % by a black cloth on a metallic support placed 2 cm above the leaf surface.

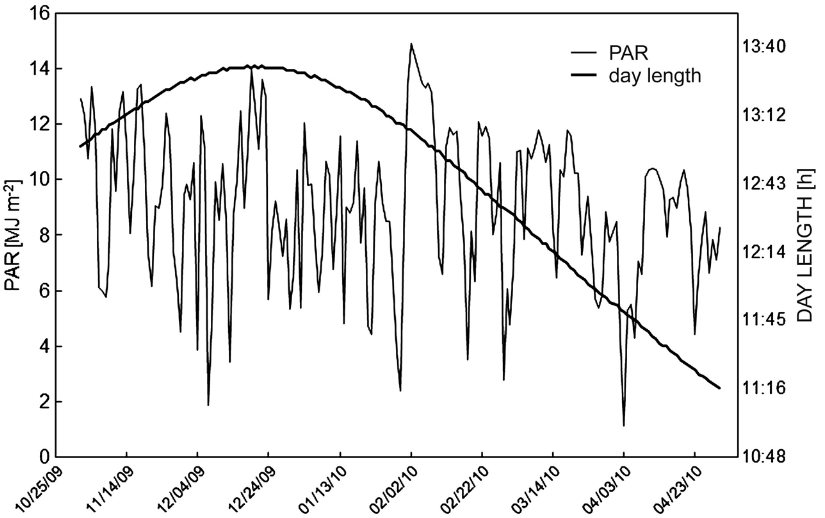

Fig. 1. The incident daily photosynthetically active radiation (PAR) and day length in the greenhouse of the São Paulo State University, Botucatu, SP, Brazil.

In the first experiment, the effect of cotton leaf age, and short and long-term shading on photosynthesis were determined. Sympodial branch leaves 15-, 30-, 45-, and 60-d-old were pre-shaded for 4 d or kept under the natural irradiance (preconditioning). By the time of evaluations, the same leaves were shaded or kept under the natural irradiance. Hence, we had four combinations: pre-shade/shade, no pre-shade/shade, pre-shade/no shade, and no pre-shade/no shade. The experimental design was a 4 × 2 factorial in complete randomized blocks (leaf age × preconditioning) with two sub-plots (the conditions at the time of assessment) and four replications. Cotton was sowed at 15-d intervals in order to obtain leaves 15-, 30-, 45-, and 60-d-old at the same plant position on the evaluation day. Leaves were labeled at unfolding, using a different plant for each leaf age. Ambient PARs reaching the leaves were 436, 491, 187 and 231 µmol m^{-2} s^{-1} for leaves 15-, 30-, 45-, and 60-d-old, respectively.

In the second experiment, we aimed at evaluating the effect of incident PAR and shading on leaf gas exchange recovery, similar to what happens after a period of cloudy weather. Treatments included preconditioning (shaded and unshaded) and PARs of 1 500, 1 200, 900, 600, 300, 100, 50, and 0 µmol m^{-2} s^{-1} during measurements. A sympodial branch leaf was labeled at unfolding and measurements were taken when it was 15-d-old. Shade was applied 4 d prior to the evaluation. Ambient PAR reaching unshaded and shaded leaves was 886 and 416 µmol m^{-2} s^{-1}, respectively. The experimental design was a complete randomized block with four replications.

Finally, to assess the effect of leaf age and PAR, the third experiment was carried out. Treatments consisted of leaf ages of 15, 30, 45, and 60 d and PARs of 1500, 1200, 900, 600, 300, 100, 50, and 0 μmol m^{-2} s^{-1}. Determinations were made in leaves of the same plant, and the ages were estimated assuming that the youngest leaf reaching 2.5 cm was 1-d-old. The experimental design was a complete randomized block with four replications.

P_N, stomatal conductance (g_s), and intercellular CO_2 concentration (c_i) were determined using a portable photosynthetic system *LI-6400* (*LI-COR*., Lincoln, NE, USA) between 9:00 and 11:00.

After running *ANOVA*, regressions were fit to data where appropriate. Curves were fitted according to the asymmetric model f = y0 + a[sqrt(x)] + bx, or the exponential model f = a[1 - exp(-bx)] using the *Sigma Plot*® software. Mean values were used to fit the curves. Treatment means were compared by LSD ($\alpha = 0.05$).

Results and discussion

The leaf age had no effect on P_N under low PAR. However, P_N was higher in 15- and 30-d-old compared with 45- and 60-d-old leaves when PAR was over 300 μmol m^{-2} s^{-1} (Fig. 2*A*). The response of P_N to PAR followed an asymptotic or exponential model-like curve, typical of C_3 plants (Pettigrew 2004). P_N increased fastly with PAR up to 700 to 800 μmol m^{-2} s^{-1}, but the complete saturation was not reached at 1 500 μmol m^{-2} s^{-1}, as it has been observed before (Zeng *et al.* 2012). The compensation radiation, when carbon fixation is equivalent to respiration (Taiz and Zeiger 2010), was significantly lower in 60-d-old leaves (9.5 μmol m^{-2} s^{-1}) compared to younger leaves (26, 24 and 19 μmol m^{-2} s^{-1} in 15-, 30- and 45 d-old leaves), similarly as reported by Constable and Rawson (1980) and Pettersen *et al.* (2010). Chlorophyll fluorescence was not measured in this study; however, previous works have shown a decreased leaf fluorescence with leaf aging (Šesták and Šiffel 1997, Čaňová *et al.* 2008). A decrease in content of chlorophyll and total proteins and of some parameters of chlorophyll fluorescence in wheat leaves with plant age was observed by Špundová *et al.* (2005). Furthermore, these authors reported that senescence is accelerated by shading as result of lipid peroxidation. In our experiments, the age was an important factor controlling P_N in cotton leaves, and the irradiance was more important for young leaves as

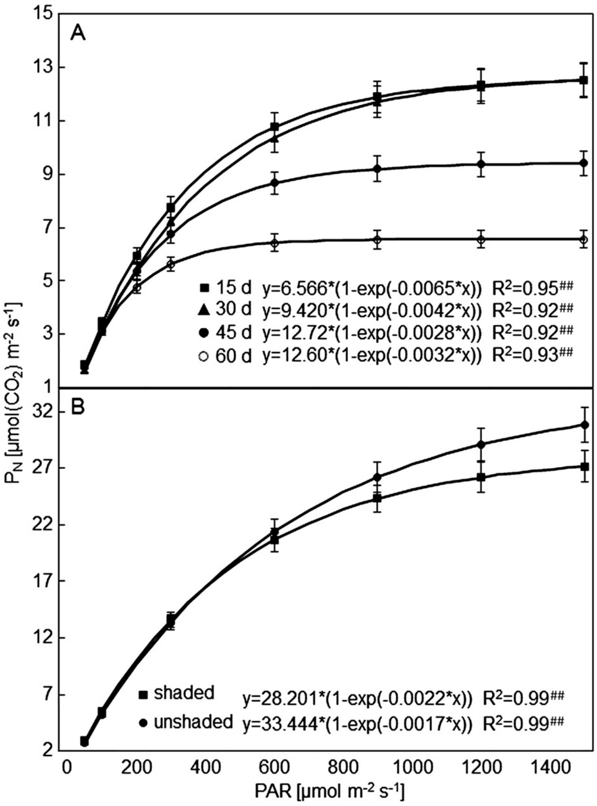

Fig. 2. *A* - The net photosynthetic rate (P_N) of cotton leaves as affected by photosynthetically active radiation (PAR) and leaf age. *B* - P_N of 15-d-old leaves as affected by incident PAR and previous shade. *Vertical bars* show SE of the mean, *n* = 4, $^{\#\#}$ - significant at 1 % probability.

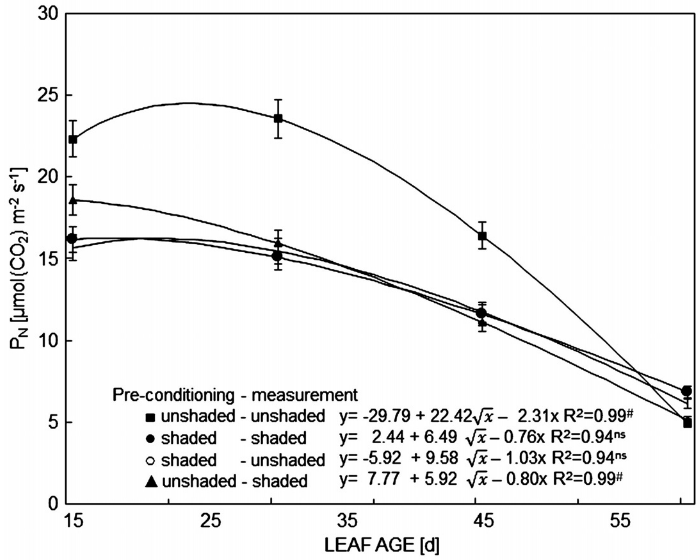

Fig. 3. P_N as affected by leaf age and shade (leaves pre-conditioned in shade for 4 d prior to the measurement and then shaded or not shaded during the measurement; control plants were kept under PAR of 500 µmol m^{-2} s^{-1}). *Vertical bars* show SE, *n* = 4, # - significant at 5 % probability, ns - non significant.

Table 1. The *P*-values for sources of variation and their interactions for net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (c_i). PAR - incident photosynthetically active radiation, shade - leaves were pre-shaded for 4 d prior to the assessment, time of measurement – leaves were shaded or kept under the natural irradiance.

	Source	P_N	g_s	c_i
Experiment 1	shade	0.02	0.62	0.21
	time of measurement	0.10	0.56	0.22
	leaf age	0.0001	0.0001	0.0001
	shade × time of measurement	0.05	0.47	0.23
	shade × leaf age	0.15	0.18	0.35
	time of measurement × leaf age	0.57	0.64	0.82
Experiment 2	shade	0.0001	0.0001	0.0001
	PAR	0.00001	0.0001	0.0001
	shade × PAR	0.01	0.16	0.07
Experiment 3	leaf age	0.0001	0.0001	0.08
	PAR	0.0001	0.02	0.0001
	leaf age × PAR	0.06	0.99	0.66

compared with mature and senescent ones (Fig. 2, Table 1).

In 15-d-old leaves either acclimated or non-acclimated to shade, P_N increased with PAR (Fig. 2*B*). However, P_N was higher ($P < 0.01$) in unshaded leaves when PAR was over 800 µmol m^{-2} s^{-1}. Thus, increasing PAR instantaneously to 1 500 µmol m^{-2} s^{-1} was not enough for the shaded leaves (acclimated at 390 µmol m^{-2} s^{-1}) to reach the same P_N as compared with the unshaded leaves, which suggests an adverse effect of shade similar to what happens after a period of cloudy weather. Zhang *et al.* (1995) also reported an adverse effect of shade in P_N recovery of *Encelia farinosa*, a C3 plant, and as a result, the electron transport rate and sugar content decreased. In the present experiment, the saturation of P_N was not reached in 15-d-old shaded or unshaded leaves under maximum PAR (1 500 µmol m^{-2} s^{-1}) used, showing that the shade, despite decreasing P_N, did not change the PAR necessary for the P_N saturation. These results confirm the observation of Sassenrath-Cole *et al.* (1996), who reported that the saturation of P_N is not achieved in 14-d-old cotton leaves at PAR of 2 000 µmol m^{-2} s^{-1}. On the other hand, Greer *et al.* (2011) reported a higher P_N of vine leaves exposed to full sunlight than those shaded to 70 %, but the saturation of P_N in shaded leaves is 45 % lower when compared with leaves under full light.

Maximum P_N in unshaded leaves was reached between 15 and 30 d (Fig. 3) and P_N decreased as the leaf age further increased. The shade played a significant role in decreasing P_N, which was less evident as leaves aged. For those leaves acclimated to shade for 4 d, there was no difference in P_N between shaded and unshaded treatments at measurement. This result shows that even after a period of shading, plants did not reestablish their leaf photosynthetic capacity (Fig. 2*B* and 3). Landivar *et al.* (2010) reported an effect of the previous history on utilization of radiation by the canopy. In other words, a leaf grown under low PAR exhibits a lower saturated P_N than a leaf developed under high PAR, what supports our results. Thirty-d-old leaves not exposed to the shade had a higher ($P < 0.01$) P_N compared with other combinations, and 45-d-old unshaded leaves had higher ($P < 0.01$) P_N compared with shaded leaves of the same age (Fig. 3). In contrast, Sassenrath-Cole *et al.* (1996) observed that P_N of cotton leaves is much more affected by leaf age than by irradiance, ranging from approximately 50 μmol(CO_2) m^{-2} s^{-1} at 15 d after leaf unfolding to 10 μmol(CO_2) m^{-2} s^{-1} at 65 d. It was observed in this experiment that leaf age affects the photosynthetic efficiency. However, PAR is also a very important factor because leaves preconditioned under the sun and subjected to shading on the measurement time decreased their photosynthetic efficiency due to decreasing incident PAR from 830 to 428 μmol m^{-2} s^{-1} compared with unshaded leaves. Self-shading and leaf aging occur concurrently in the plant canopy and there is little PAR available for older leaves at the bottom of the plant canopy. The results obtained in the present experiment corroborate findings by Wullschleger and Oosterhuis (1990), who showed that young leaves at the top of the canopy receiving PAR of 1 700 μmol m^{-2} s^{-1} fix CO_2 at a rate of 32.8 μmol m^{-2} s^{-1}, whereas leaves in the lower third of the canopy received PAR of 350 μmol m^{-2} s^{-1} and fix only 10.9 μmol m^{-2} s^{-1} of CO_2.

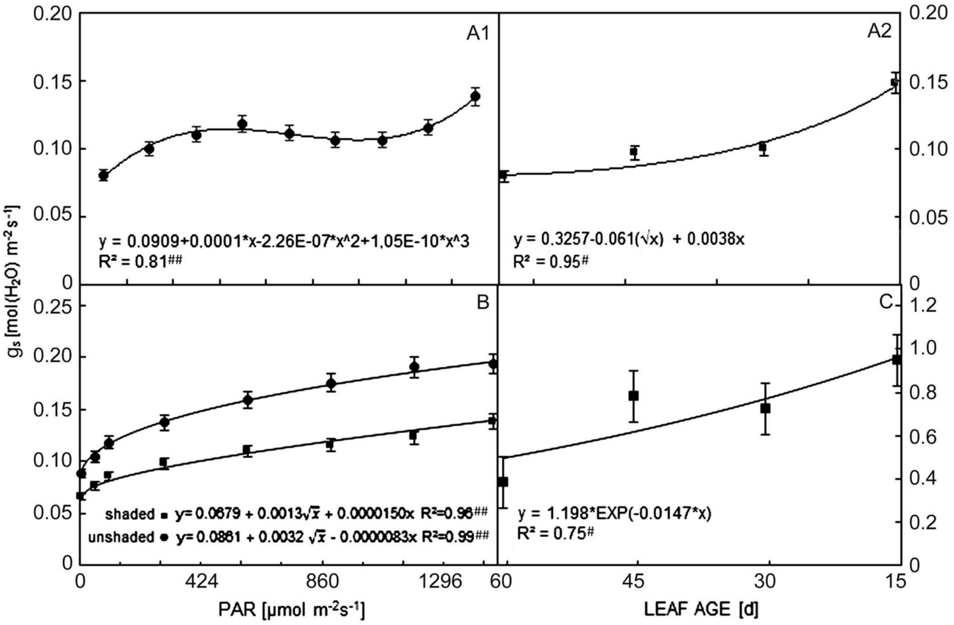

Fig. 4. Stomatal conductance (g_s) as affected by leaf age, shade, and incident PAR. *A1* - the mean of eight PAR values – experiment 3, *n* = 16; *A2* - the mean of four leaf ages – experiment 3, *n* = 16; *B* - 15-d-old leaves were shaded for 4 d – experiment 2, *n* = 4; *C* - the mean of four shade treatments – experiment 1, *n* = 16). *Vertical bars* show SE. $^{\#}$ and $^{\#\#}$ significant at 5 and 1 % probability, respectively.

Stomatal conductance (g_s) also decreased ($P < 0.01$) with leaf aging ($R^2 = 0.95$), and PAR accounted for about 80 % of g_s response ($R^2 = 0.81$) (Fig. 4*A2* and *A1*) when leaves of all ages were considered. For young cotton leaves, Sun *et al.* (2009) also reported an increased g_s as a response to an increased irradiance. Contrary to a maximum g_s at 15-d-old leaves observed in this work, previous research showed a maximum g_s in cotton leaves at 25 d (Constable and Rawson 1980) and 21 d (Kakani *et al.* 2004), and these differences may be attributed to different cultivars.

In young leaves (15-d-old), g_s increased in both unshaded and shaded leaves as PAR increased (Fig. 4*B*). Similar to what was observed for P_N (Fig. 2*B*), g_s increased with PAR, but it was significantly lower in shaded leaves and did not recover to the levels of unshaded leaves. A previous study also observed that shading (120 μmol m^{-2} s^{-1}) reduced g_s of cotton leaves as compared to unshaded (1 950 μmol m^{-2} s^{-1}) (Petersen *et al.* 1991). Thus, leaves grown under shade do not reach the same g_s as unshaded leaves, which was also observed in the present experiment. The effect of shading on g_s may be associated with the leaf abscisic acid (ABA) content, because the content of ABA is expected to be reduced with the increasing irradiance of mesophyll, thus enhancing stomatal opening (Farquhar and Sharkey 1982).

Leaf aging resulted in decreasing g_s (Figs. 4*A2* and 4*C*). However, our results contrast with Constable and Rawson (1980), where g_s was maximum at 25 d. Closing stomata with further ageing may be regulated by ABA as ABA content increases in leaf tissues as they age (Jibran *et al* 2013).

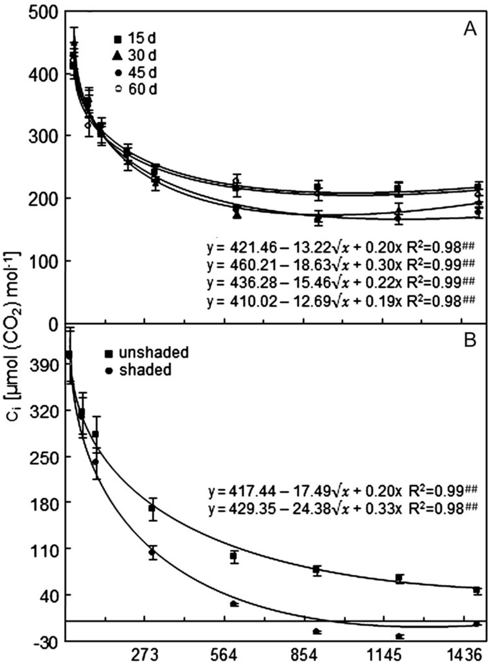

Fig. 5. The intercellular CO_2 concentration (c_i) of cotton leaves as affected by PAR and leaf age (*A,* experiment 1); PAR and shade (*B,* 15-d-old leaves were shaded or unshaded for 4 d prior to the measurement – experiment 2). *Vertical bars* show SE, *n* = 4, ## significant at 1% probability.

The c_i in the cotton leaves decreased with increasing PAR irrespective of age (Fig. 5*A*), as also reported by Zhou *et al.* (2009). A c_i decrease in sub-stomatal cavities is a result of increased carbon fixation or CO_2 conductance in the mesophyll (Messinger *et al.* 2006). In 15-d-old leaves, increased PAR reduced c_i, and the decrease was higher ($P < 0.01$) in shaded leaves than in unshaded ones (Fig. 5*B*). Shaded leaves had a lower c_i response to PAR, possibly due to lower g_s (Fig. 4*B*). In contrast, the decrease in c_i in unshaded leaves resulted from a higher carbon fixation as response to increased PAR (Fig. 2*B*). These results are consistent with previous observations in cotton, where c_i increases in shaded leaves reflecting the reduced P_N (Zhao and Oosterhuis 1998b). The average decrease in atmospheric CO_2 (c_a) in sub-stomatal cavities (c_a - c_i) in herbaceous dicotyledonous leaves under saturated irradiance is 125 ± 5 µmol mol^{-1} when c_a is 360 µmol mol^{-1} (Warren 2008). Although the saturation of P_N was not reached, c_i at the highest PAR (1 500 µmol m^{-2} s^{-1}) was 45 µmol mol^{-1} in unshaded leaves and 20 µmol mol^{-1} in shaded leaves showing that the shade restricted CO_2 flux into the sub-stomatal cavities (Fig. 4*B*).

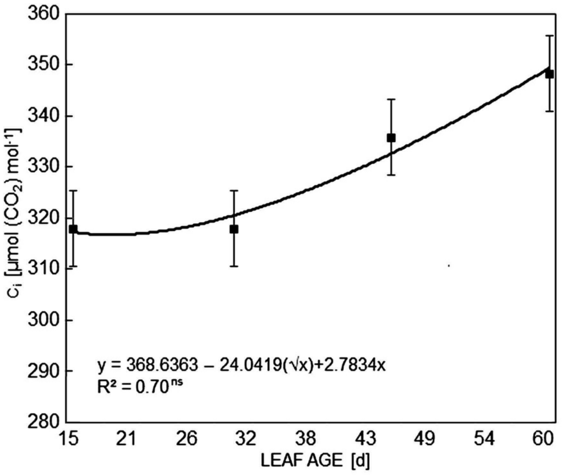

Fig. 6. The intercellular CO_2 concentration of cotton leaves as affected by leaf age (mean of preconditioning and shade at measurement – experiment 1*). Vertical bars* show SE, *n* = 16. ^{ns} - non-significant.

Leaf aging increased c_i, but it was not affected by pre-shading (Fig. 6). Previous studies have shown that leaf age has little effect on c_i (Constable and Rawson 1980), but a high irradiance can decrease c_i by increasing P_N (Smith and Longstreth 1994, Xu and Hsiao 2004). A high c_i suggests a limitation in carbon fixation as observed in the present experiment (Figs. 3 and 6) as well as in a previous study where a c_i increase is associated with a decreased P_N in senescent maize leaves (Lu and Zhang 1998).

The objective of this study was to evaluate the effects of leaf age, incident PAR, and shading, alone or in combination, on cotton leaf gas exchange. We hypothesized that the effect of shading could be more important than the effect of leaf aging. The short-term shade decreased P_N, mainly in young leaves (15- and 30-d-old). These leaves did not re-establish their P_N under increasing PAR. The response of older leaves to PAR was lower when compared to young leaves suggesting that self-shading is not primarily responsible for the decreased P_N in the old cotton leaves. This finding improves the understanding of changes in P_N in leaves of cotton grown in population of high density, which may help cotton growers.

References

Bauer, P.J., Sadler, E.J., Frederick, J.R.: Intermittent shade effect on gas exchange of cotton leaves in the humid southeastern USA. - Agron. J. **89**: 163-166, 1997.

Brodrick, R., Bang, M.P., Milroy, S.P., Hammer, G.L.: Physiological determinants of high yielding ultra-narrow row cotton: canopy development and radiation use efficiency. - Field Crops Res. **148**: 86-94, 2013.

Čaňová, I., Ďurkovič, J., Hladká, D.: Stomatal and chlorophyll fluorescence characteristics in European beech cultivars during leaf development. - Biol. Plant. **52**: 577-581, 2008.

Constable, G., Rawson, H.: Effect of leaf position, expansion and age on photosynthesis, transpiration and water use efficiency of cotton. - Funct. Plant Biol. **7**: 89-100, 1980.

Dauzat, J., Clouvel, P., Luquet, D., Martin, P.: Using virtual plants to analyse the light-foraging efficiency of a low-density cotton crop. - Ann. Bot. **101**: 1153-1166, 2008.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Greer, D.H., Weedon, M.M., Weston, C.: Reductions in biomass accumulation, photosynthesis *in situ* and net carbon balance are the costs of protecting *Vitis vinifera* 'Semillon' grapevines from heat stress with shade covering. - AoB Plants doi:10.1093/aobpla/plr023**,** 2011.

Guinn G., Brummett D.L.: Leaf age, decline in photosynthesis, and changes in abscisic acid, indole-3-acetic acid, and cytokinin in cotton leaves. - Field Crops Res. **32**: 269-275, 1993.

Jibran, R., Hunter, D., Dijkwel, P.: Hormonal regulation of leaf senescence through integration of developmental and stress signals. - Plant mol. Biol. **82**: 547-561, 2013.

Kakani, V.G., Reddy, K.R., Zhao, D., Gao, W.: Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation and carbon dioxide. - Physiol. Plant. **121**: 250-257, 2004.

Landivar, J.A., Reddy, K.R., Hodges, H.F.: Physiological simulation of cotton growth and yield. - In: Stewart, J., Oosterhuis, D.M., Heitholt, J.J., Mauney, J. (ed.): Physiology of Cotton. Pp. 319-331. Springer, New York 2010.

Lu, C., Zhang, J.: Modifications in photosystem II photochemistry in senescent leaves of maize plants. - J. exp. Bot. **49**: 1671-1679, 1998.

Messinger, S.M., Buckley, T.N., Mott, K.A.: Evidence for involvement of photosynthetic processes in the stomatal response to CO_2. - Plant Physiol. **140**: 771-778, 2006.

Milroy, S.P., Bange, M.P., Sadras. V.O.: Profiles of leaf nitrogen and light in reproductive canopies of cotton (*Gossypium hirsutum*). - Ann. Bot. **87**: 325-333, 2001.

Petersen, K.L., Moreshet, S., Fuchs, M.: Stomatal responses of field-grown cotton to radiation and soil moisture. - Agron J. **83**: 1059-1065, 1991.

Pettersen, R.I., Torre, S., Gislerød, H.R.: Effects of leaf aging and light duration on photosynthetic characteristics in a cucumber canopy. - Sci. Hort. **125**: 82-87, 2010.

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

Saeki, T.: Light relations in plant communities. - In: Evans, L.T. (ed.): Environmental Control of Plant Growth. Pp. 79-94. Academic Press, New York - London 1963.

Sassenrath-Cole, G.F., Lu, G., Hodges, H.F., Mckinion, J.M.: Photon flux density versus leaf senescence in determining photosynthetic efficiency and capacity of *Gossypium hirsutum* L. leaves. - Environ. exp. Bot. **36**: 439-446, 1996.

Šesták, Z., Šiffel, P.: Leaf-age related differences in chlorophyll fluorescence. - Photosynthetica **33**: 347-369, 1997.

Smith, J.E., Longstreth, D.J.: Leaf expansion and carbon assimilation in cotton leaves grown at two photosynthetic photon flux densities. - Amer. J. Bot. **81**: 711-717, 1994.

Špundová, M., Slouková, K., Hunková M., Nauš, J.: Plant shading increases lipid peroxidation and intensifies senescence-induced changes in photosynthesis and activities of ascorbate peroxidase and glutathione reductase in wheat. - Photosynthetica **43**: 403-409, 2005.

Sun, C.X., Qi, H., Hao, J.J., Miao, L., Wang, J., Wang, Y., Liu, M., Chen, L.J.: Single leaves photosynthetic characteristics of two insect-resistant transgenic cotton (*Gossypium hirsutum* L.) varieties in response to light. - Photosynthetica **47**: 399-408, 2009.

Taiz, L., Zeiger, E. (ed.): Plant Physiology. Fifth Ed. - Sinauer Associates, Stamford 2010.

Warren, C.R.: Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO_2 transfer. - J. exp. Bot. **59**: 1475-1487, 2008.

Wells, R.: Light and the cotton plant. - In: Oosterhuis, D. (ed.): Stress Physiology in Cotton. Pp. 73-83. The Cotton Foundation, Cordova 2011.

Wells, R.: Response of leaf ontogeny and photosynthetic activity to reproductive growth in cotton. - Plant Physiol. **87**: 274-279. 1988.

Wullschleger, S.D., Oosterhuis, D.M.: Photosynthetic and respiratory activity of fruiting forms within the cotton canopy. - Plant Physiol. **94**: 463-469, 1990.

Xu, L.-K., Hsiao, T.C.: Predicted *versus* measured photosynthetic water-use efficiency of crop stands under dynamically changing field environments. - J. exp. Bot. **55**: 2395-2411, 2004.

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

Zhang, H., Sharifi, M., Nobel, P.: Photosynthetic characteristics of sun *versus* shade plants of *Encelia farinosa* as affected by photosynthetic photon flux density, intercellular CO_2 concentration, leaf water potential, and leaf temperature. - Funct. Plant Biol. **22**: 833-841, 1995.

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

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

Zhou, X., Li, Y., Zhang, X., Shen, S.: Difference of photosynthetic characteristics of different height leaves in the cotton flower-boll development phase in Huaibei area. - Trans. atm. Sci **32**: 673-76, 2009.