# **1** Different Light Radiation Intensities on Cotton: A

# 2 **Physiological Approach**

3

## 4 Abstract

The luminosity and the temperature are factors that act directly in the 5 photosynthetic process, where an elevation of the luminous intensity can cause 6 a reduction of the assimilation of carbon, which consequently lowers the 7 development of the cotton. The objective of this work was to assess the 8 response of physiological parameters of cotton when subjected to different 9 artificial light intensities. Two varieties of cotton IMA5801B2RF and IACRDN, 10 were interacting with five artificial light intensities: 0 (control): 500: 1000; 1500 11 and 2000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> of photosynthetically active radiation provided by LED 12 bulbs. The experiment was set in a randomized complete block design using a 13 2x5 factorial scheme. The variables measured were the rate of CO<sub>2</sub> 14 assimilation, transpiration, stomatal conductance, inner CO<sub>2</sub> concentration in 15 the substomatic chamber, and efficient use of water (for which a portable device 16 17 of gas exchange was used). The cotton varieties responded positively to different luminous intensities until reaching the point of maximum saturation 18 between 1400 and 1600 µmol m<sup>-1</sup> s<sup>-1</sup> of light, which provided a better rate of 19 CO<sub>2</sub> assimilation, concentration of CO<sub>2</sub> in the substomatic chamber, and 20 efficient use of water. Leaf transpiration and stomatal conductance showed a 21 positive linear response with increasing light intensity. The ideal luminous 22 intensity for the use of Infra-Red Gas Analyzer - IRGA was 1500 µmol m<sup>-1</sup> s<sup>-1</sup> for 23 the tested cotton varieties. 24

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26 Keywords: Gossypium L .; brightness; CO<sub>2</sub> assimilation; photosynthesis rate

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## 28 Introduction

29 Cotton (*Gossypium* L.) belongs to the Malvaceae family. It is cultivated 30 as a fiber source for the production of fabrics and for its seeds that produce 31 linoleic and linolenic oils that are used in the cosmetics or animal feed industry. 32 Therefore, it is an important crop for the Brazilian agricultural scenario, since it 33 makes an alternative in crop rotation in the production of large crops such as corn and soybeans. However, cotton can suffer interference during its
developmental stages due to climatic factors such as water stresses, pests and
diseases and light intensity, especially in the establishment and reproduction
phases [1].

Light is the primary source of energy related to photosynthesis and 38 morphogenetic phenomena, and is one of the main factors that influence plant 39 40 growth and development [2, 3, 4]. Nevertheless, increase in light intensity can 41 reduce the photosynthetic activity through photoinhibition, and this response 42 can be variable between plant species and varieties [5, 6]. The luminous intensity and the temperature are factors that can limit the photosynthetic 43 process and also contribute to the reduction of the carbon acquisition, 44 consequently causes a reduction in rate of plant growth [7]. 45

The plants when subjected to medium intensity light show less transpiration when compared to plants that are exposed to more intense light intensity, that is, less light is a limiting factor for leaf transpiration [8]. The importance of light intensity in the physiological process of the plant, is evidenced in its direct link in the activation of enzymes related to carbon fixation and in the control in the opening and closing in the stomatal cleft [9, 10, 11].

52 It is important to emphasize that the understanding in the balance of intensity levels and the duration of exposure to light that plants can be 53 subjected to makes it an important factor to understand the responses of plants 54 to varying light stress. When exposed to direct low-intensity radiation, the plants 55 56 become more efficient in carrying out their photosynthesis, since the process is 57 started in a gradual way, which does not compromise the pathways of the electrons by the photosystems. But with the increase of this intensity of photons 58 that affect the leaves, the plants present an elevation in the photolysis of the 59 water, which results in a saturation of electrons, causing a reduction in the rate 60 61 of assimilation of  $CO_2$  and in the efficient use of water [12, 13].

This work had as objective to know the response of selected physiological parameters to different intensities of light radiation on cotton crop.

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65 Material and Methods

The experiment was carried out in December 2018, at the Paulista Agribusiness Technology Agency (APTA), located in the city of Adamantina, São Paulo State, latitude 21°40'24.024" S and longitude 51°8'31.088" W, at an altitude of approximately 420 m. The climate of the region is characterized as Aw according to Köppen, with rainy summers and dry winters; with an annual average temperature of 22.1°C and 1204 mm of rain accumulated in the year.

The experiment was carried out in randomized blocks, in a factorial scheme of 2x5, including 2 varieties of cotton; IMA5801B2RF and IAC-RDN, interacting with 5 densities of light: 0 (control); 500; 1000; 1500 and 2000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> of photosynthetically active radiation (PAR) provided by LED bulbs.

The area soil was classified as red-yellow Latosol [14] and its chemical attributes are presented in Table 1.

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 Table 1: Chemical attributes of the soil of the experiment area at the time of sowing of cotton

pН		OM	Р	Κ	Ca Mg	H+AI	AI	SB	CEC	V%	m%
(CaCl <sub>2</sub> )		(g dm⁻³)	mg dm <sup>-</sup>								
			3	Ę			mi	mol <sub>c</sub> d	m <sup>-3</sup>		
4.6		12.0	26.0	2.9	8.0 4.0	20.0	1.0	14.9	34.9	43.0	6.0
	<b>6</b> 1	1.404				0/	<u> </u>				

SB: Sum of bases; V%: Saturation per bases; m%: Saturation per aluminum; CEC: Cation exchange capacity.

Each block consisted of five rows of five meters in length, spaced 0.9 m 81 between rows, and distance between cotton seeds at sowing was 25 cm 82 corresponding to 45 thousand plants per hectare. The soil was fertilized as per 83 the needs of cotton culture [15]. During the experiment, the cotton was watered 84 until the soil reached field capacity, and the phytosanitary treatments of the crop 85 were done using dose Thiamethoxam 250 mL ha<sup>-1</sup>; Imidacloprid 355 mL ha<sup>-1</sup> 86 and Lufenuron 500 mL ha<sup>-1</sup> of the with a syrup volume of 150 L ha<sup>-1</sup> with single 87 application. 88

Thirty days after the sowing, five plants were randomly selected within each replicate, where four readings were performed on the fully expanded leaves from the apex of the plant, totaling 20 readings for each light intensity for the different cotton varieties. The following parameters were measured: rate of CO<sub>2</sub> assimilation (A); transpiration (E); stomata conductance (GS); inner CO<sub>2</sub>

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concentration in the substomatic chamber (Ci), with 380 ppm of CO<sub>2</sub>, under
28°C temperature of chamber, a portable device of gas exchange was used
(Infra-Red Gas Analyzer - IRGA, ADC BioScientific Ltd, model LC-Pro); and
efficient use of water (EUW) by applying the following arithmetic formula:

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$$EUW = \frac{A}{E}$$

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All variables were subjected to the analysis of variance for all parameters measured. Means values were subjected to Scott & Knott Test [16]. Analyses of regression were performed for a better understanding of the relationships between each of the  $CO_2$  assimilation rate, transpiration of cotton leaf, stomata conductance, and internal  $CO_2$  concentration in the substomatic chamber and to the intensities of artificial light, in which their standards were tested: linear, quadratic and cubic using the statistic program R [17].

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### 108 **Results**

There was no difference between the varieties in the transpiration (E) and stomata conductance (GS) when the cotton was exposed to different light intensities (Table 2). However, the IAC-RDN variety showed a greater mean in the internal CO<sub>2</sub> concentration in the substamatic chamber (Ci) with a difference of 2.34% more in relation to IMA5801B2RF.

IMA5801B2RF showed higher mean values for CO<sub>2</sub> assimilation (A) and
 water efficiency (EUW), 4.68% and 5.79% more, respectively, in relation to the
 IAC-RDN variety (Table 2).

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Table 2: Mean values of rate of  $CO_2$  assimilation (A); transpiration (E); stomata conductance (GS); inner  $CO_2$  concentration in the substomatic chamber (Ci), and the efficient use of water (EUW)  $H_2O^{-1}$ ) and analysis of variance of the cotton regressions when exposed to different intensities of light radiation

Variety (V)	А	A E		Ci	EUW			
	$(\mu mol CO_2 m^{-2} s^{-1})$	$(mmol H_2O m^{-2} s^{-1})$	(mol $H_2O m^{-2} s^{-1}$ )	(µmol mol <sup>-1</sup> )	$(mol CO_2 mol H_2O^{-1})$			
IMA5801B2RF	16.66a	3.48	0.53	275.63b	4.49a			
IAC-RDN	15.88b	3.45	0.53	282.24a	4.23b			
CV%	12.07	11.54	26.20	6.21	14.38			
OM	16.27	3.46	0.53	278.93	4.36			

F(Variety (V))	8.04**	0.	28Ns	0.06Ns		7.28**	8.47**
F(Radiation (R))	1320.22**	84	4.09**	42.27**	,	639.20**	954.15**
F (V x R)	5.36**	0.	76Ns	1.73Ns		4.06**	4.36**
	VF	DF		Regress	ions mio	dle square	
	Radiation	4	2394.8730	26.7034	1.7038	137064.375	264.5401
IMA5801B2RF	Residue	96	2.8655	0.1641	0.0201	264.7738	0.3051
	Regression	1	Q**	L**	L**	Q**	Q**
	Radiation	4	1628.0518	24.4440	1.5699	88253.0035	188.8543
IAC-RDN	Residue	96	5.7066	0.1602	0.0192	378.7123	0.5611
	Regression	1	Q**	L**	L**	Q**	Q**

CV: Coefficient of variation. OM: Overall mean. F: value of F calculated in the analysis of variance; Ns p=0.05; \*0.01  $\leq$ p < 0.05; \*\*p < 0.01. The averages in the column followed by the same letter do not differ statistically from each other. The Scott & Knott test was applied at a 5% probability level. Ns- p  $\geq$  0.05; \*0.01  $\leq$  p < 0.05; \*\* p < 0.01. VF: Variation factor; DF: Degrees of freedom. L: polynomial of 1st degree. Q: polynomial of 2nd degree.

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119 When the light intensities were taken into account, the varieties 120 responded in a similar way for all the parameters evaluated (Table 2). The 121 varieties presented a positive quadratic response to the CO<sub>2</sub> assimilation rate, 122 (Figure 1), where the IMA5801B2RF variety presented a maximum point up to 123 1521 µmol m<sup>-2</sup> s<sup>-1</sup> while the IAC-RDN variety had a maximum point of 1673 124 µmol m<sup>-2</sup> s<sup>-1</sup>.

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Fig 1. CO<sub>2</sub> assimilation rate (A) of cotton varieties IMA5801B2RF (1) and IAC-RDN (2) exposed to different intensities of light radiation

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While there was an increase in light intensity, the cotton varieties presented a positive linear response to the transpiration parameter of the leaf (E) as shown in Figure 2.



# Fig. 2. Transpiration of cotton leaf (E) from varieties IMA5801B2RF (1) and IAC-RDN (2) exposed to different intensities of light radiation

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132 Similarly, the varieties exhibited a positive response to the increase in

light intensity for stomata conductance (GS) (Figure 3).

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In contrast, the internal  $CO_2$  concentration in the sub-static chamber (Ci) of the cotton varieties presented negative quadratic responses to increases in light intensity, where a minimum point of 1385 µmol m<sup>-2</sup> s<sup>-1</sup> was observed in the variety IMA5801B2RF and 1528 µmol m<sup>-2</sup> s<sup>-1</sup> for the IAC-RDN variety, as shown in Figure 4.



Fig. 4. Internal CO<sub>2</sub> concentration in the substomatic chamber (Ci) of cotton varieties IMA5801B2RF (1) and IAC-RDN (2) exposed to different intensities of light radiation

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With the increase in the intensity of the light radiation on the leaves, the cotton varieties presented a quadratic positive response to the parameter EUW (water efficient use) (Figure 5), where the maximum points were 1375  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> for the variety IMA5801B2RF and 1489  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> for IAC-RDN.





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Negative correlations were observed between the internal CO<sub>2</sub> concentration in the substomatic chamber (Ci) interacting with leaf transpiration (E), stomatal conductance (GS), rate of assimilation of CO<sub>2</sub> (A), and water use efficiency (EUW) as shown in Table 3.

	variables of ootion when submitted to anterent light intensity									
	Ci	E	GS	A						
Е	-0.5733**									
GS	-0.3943**	0.94156**								
А	-0.9432**	0.79094**	0.64496**							
EUW	-0.9955**	0.61386**	0.44222**	0.96071**						

Table 3: Pearson correlation coefficient *r* values among the analyzed variables of cotton when submitted to different light intensity

Ns  $p \ge 0.05$ ; \*0.01  $\le p < 0.05$ ; \*\* p < 0.01. rate of CO<sub>2</sub> assimilation (A), transpiration (E), stomata conductance (GS), inner CO<sub>2</sub> concentration in the substomatic chamber (Ci), and the efficient use of water (EUW).

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### 155 **DISCUSSION**

The plant can respond in different ways to a change of the environment 156 in which it was inserted, where the luminosity is restrictive to the development of 157 the plant, since the quality and the luminous intensities that affect the leaves 158 alter the responses in the PSII and PSI complexes of the photosystem. This can 159 cause changes in the photolysis of the water, which consequently results in the 160 release of electrons during photosynthesis due to the increase or restriction of 161 the photons that are affecting the plant [13]. In this study, the ideal intensity 162 observed was approximately 1500 µmol m<sup>-1</sup> s<sup>-1</sup> light falling on the leaves of the 163 164 cotton plant.

165 It is worth noting that, even at different periods of the day, a variation 166 occurs in the intensity of light energy, which influences the CO<sub>2</sub> assimilation rate 167 of the leaves [18] demonstrating this phenomenon that occurs naturally during 168 the day.

The significant negative correlation between the internal concentration of 169 170 CO<sub>2</sub> in the sub-static chamber (Ci) and the other variables as shown in Table 3 was already expected, since the internal concentration of CO<sub>2</sub> is reduced while 171 the carbon fixation in the dry matter of the cotton occurs via Rubisco molecule, 172 which results in the elevation of the  $CO_2$  assimilation rate (A). In this way, this 173 interaction can be verified when one observes Figure 1 and Table 2, where the 174 absence of light on the leaves caused a negative assimilation rate (A), while the 175 internal CO<sub>2</sub> concentration was high (Figure 4). And with the increase in light 176 177 radiation, the stomata were opened, consequently causing an increase in the

transpiration rate (E) (Figure 2) and the stomata conductance (GS) (Figure 3) 178 and thus led to a reduction in concentration (Ci) due to a possible dilution effect, 179 where CO<sub>2</sub> at high internal concentrations is released to the environment due to 180 the stomatal opening and its fixation in the dry mass [13]. Again, the 181 understanding of these responses regarding leaf water loss with increase in the 182 luminous intensity is important in the determination of the point of maximum 183 184 response of this variable. This becomes an important tool in the decision 185 making in the cotton cultivation, since it can guarantee a better understanding of 186 the water availability requirements.

It is worth mentioning that the understanding of the mechanism of opening and closing the stomatal cleft can be compromised or enhanced with nutritional stress factors (Table 1), and the availability of  $H_2O$  in the soil-plantatmosphere system [10, 11] and even internal morphology of the leaves of each species and variety [3, 4, 5, 6]. As previously mentioned, stomata conductance presented a positive correlation with the other variables (Table 3).

The positive correlation between the CO<sub>2</sub> assimilation rate (A) interacting 193 with the use of leaf transpiration (E) was already expected, since the 194 195 relationship between these two variables yields the efficient use of water 196 (EUW), which was elevated with the increase of light radiation between 1300 and 1500  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> (Figure 5). When gas exchange occurs through the 197 stomatal cleft, the plant needs a hydrostatic pressure (Kleaf) to efficiently use 198 water (EUW) in the photosynthetic system, where water stress directly 199 influences development in different plant species in the initial phase [5, 11, 7]. 200 201 This showed that the light intensity influenced positively only until its saturation as pointed out earlier. This saturation of light caused an increase in the 202 photolysis of the water which might have led to the saturation of electrons in the 203 photosystem. 204

Thus, more in-depth studies are needed on the relationship between these variables, since species and varieties present different responses between them.

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### 209 CONCLUSION

The two cotton varieties responded positively under different light intensities up to the maximum saturation point between 1400 and 1600 μmol m<sup>-</sup> <sup>1</sup> s<sup>-1</sup> of light. This provided a better rate of assimilation of CO<sub>2</sub> (A), concentration of CO<sub>2</sub> in the substamatic chamber (Ci), and efficient use of water (EUW).Leaf transpiration (E) and stomatal conductance of the cotton showed a positive linear response with increasing light intensity.The ideal luminous intensity for the use of Infra-Red Gas Analyzer - IRGA was 1500  $\mu$ mol m<sup>-1</sup> s<sup>-1</sup> in the cotton crop.

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### 219 COMPETING INTERESTS DISCLAIMER

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Authors have declared that no competing interests exist. The products 221 used for this research are commonly and predominantly use products in 222 our area of research and country. There is absolutely no conflict of 223 224 interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for 225 the advancement of knowledge. Also, the research was not funded by the 226 producing company rather it was funded by personal efforts of the 227 authors. 228

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#### 230 **REFERENCES**

 Echer FR, Zanfolin PRL, Moreira ACM, Santos ACP, Gorni PH. Root growth and carbohydrate partitioning in cotton subjected to shading in the initial phase. Ciência Rural. 2016; 49(1):1-8. <u>http://dx.doi.org/10.1590/0103-</u> 8478cr20180749

- 235 2. Holt JS. Plant response to light: a potencial tool for weed
  236 management. Weed Science. 1995; 43: 474-482.
- Stewart, JJ, Polutchko SK, Adams Iii, WW, Cohu CM, Coleman A, Wenzl 237 3. CA, Demmig-Adams B. Light, temperature and tocopherol status influence 238 foliar vascular anatomy function 239 and leaf in Arabidopsis thaliana. Physiologia Plantarum. 2017; 160(1):98-110. 240 http://dx.doi.org/10.1111/ppl.12543 241
- 4. Feldman AB, Leung H, Baraoidan M, Elmido-Mabilangan A, Canicosa I,
  Quick WP, Sheehy J, Murchie EH. Increasing leaf vein density via
  mutagenesis in rice results in an enhanced rate of photosynthesis, smaller

- cell sizes and can reduce interveinal mesophyll cell number. Frontiers In
  Plant Science. 2017; 8:1-10. <u>http://dx.doi.org/10.3389/fpls.2017.01883</u>
- 5. Xiong D, Douthe C, Flexas J. Differential coordination of stomatal
  conductance, mesophyll conductance, and leaf hydraulic conductance in
  response to changing light across species. Plant, Cell & Environment. 2018;
  41(2):436-450. <u>http://dx.doi.org/10.1111/pce.13111</u>
- Rockwell FE, Holbrook NM. Leaf Hydraulic Architecture and Stomatal
   Conductance: A Functional Perspective. Plant Physiology. 2017;
   174(4):1996-2007. http://dx.doi.org/10.1104/pp.17.00303
- Araújo SAC, Deminicis BB. Photoinhibition of the Photosynthesis. Brazilian
   Journal of Biosciences. 2006; 7(4): 463-472. In Portuguese
- 8. Vieira TO, Degli-Esposti MSO, Souza GM, Rabelo GR, Vitória AP. 256 257 Photoacclimation capacity in seedling and sapling of Siparuna guianensis (Siparunaeae): irradiance 258 Response to gradient in tropical 2015; 259 forest. Photosynthetica. 53(1):11-22. http://dx.doi.org/10.1007/s11099-015-0073-x 260
- Teixeira MC, Vieira TO, Almeida TCM, Vitória AP. Photoinhibition in Atlantic
   Forest native species: short-term acclimative responses to high
   irradiance. Theoretical And Experimental Plant Physiology. 2015; 27(3 4):183-189. <u>http://dx.doi.org/10.1007/s40626-015-0043-5</u>
- 10. Bellasio C, Quirk J, Buckley TN, Beerling DJ. A dynamic hydro-mechanical 265 model of and biochemical stomatal conductance for C4 266 photosynthesis. Plant Physiology. 2017: 175(1):104-119. 267 268 http://dx.doi.org/10.1104/pp.17.00666
- 11. Li Y, Li H, Li Y, Zhang S. Improving water-use efficiency by decreasing
   stomatal conductance and transpiration rate to maintain higher ear
   photosynthetic rate in drought-resistant wheat. The Crop Journal. 2017;
   5(3):231-239. <u>http://dx.doi.org/10.1016/j.cj.2017.01.001</u>
- 12. Atroch EMAC, Soares AM, Alvarenga AA, Castro EM. Growth, chlorophyll
  content, biomass distribution and anatomical characteristics of young plants
  of *Bauhinia forficata* link submitted to shading. Ciência e Agrotecnologia.
  2001; 25(4):853-862. In Portuguese
- 13. Taiz L, E Zeiger. Fisiologia vegetal. 5. ed. Porto Alegre: Artmed. 2013;
  918p.

- 279 14. Embrapa Empresa Brasileira de Pesquisa Agropecuária. Sistema
   280 brasileiro de classificação de solos. 3.ed. Brasília. 2013; 353p.
- 15. Raij B, Cantarella H, Quaggio JÁ, Furlani AMC. Recomendações de
  adubação e calagem para o Estado de São Paulo. 2.ed. Campinas: IAC.
  1996; 285p.
- 16. Banzatto DA, Kronka SN. Experimentação Agrícola. 4.ed. Funep. 2013;
  285 237p.
- 17. R Development Core Team. R: A language and environment for statistical
   computing. R Foundation for Statistical Computing, Vienna, Austria. 2009.
   ISBN 3-900051-07-0, URL <a href="http://www.R-project.org">http://www.R-project.org</a>
- 18. Kim S, Nusinow DA, Sorkin ML, Pruneda-Paz J, Wang X. Interaction and
- 290 regulation between lipid mediator phosphatidic acid and circadian clock
- regulators in Arabidopsis. The Plant Cell. 2019; 1-58.

292 <u>http://dx.doi.org/10.1105/tpc.18.00675</u>