

## Effect of continuous and intermittent shade on carbohydrate accumulation and root growth in cotton

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**ABSTRACT:** Light restriction is one of the environmental factors that most limits cotton (*Gossypium hirsutum* L.) growth and production in tropical regions, due to the plant exposure to cloudy days with low radiation availability. This study evaluated the initial development of cotton root growth under intermittent shade intervals of 2 (2-d), 4 (4-d), and 8 (8-d) days, continuous shade and no-shade (control) treatments, using a black screen to reduce light intensity by 50%. Continuous shade reduced the total root length by 30% compared to the control. Decreases in root length were verified on the thinnest roots (0-0.5 mm) and were approximately 47% in the shaded and 2-d shaded treatments, 33.8% with an 8-d shade interval, and 11% with a 4-d shade interval. Plants shaded continuously and under the 2-d-shade interval showed higher height, but fewer leaves. Continuous shading and 4-d shade interval decreased the plant biomass and the carbohydrate accumulation on leaves and squares. It was concluded that continuous or intermittent 4-d shade reduces carbohydrate content on leaves and stems, continuous shading reduces cotton root length, and the root length of thinner roots (0-0.5 mm) is affected by continuous or 2-d intermittent shade.

**Key words:** low radiation; reproductive dry weight; root diameter; root length

## Efeito do sombreamento contínuo e alternado sobre o acúmulo de carboidratos e crescimento radicular do algodoeiro

**RESUMO:** A restrição de luz é um dos fatores ambientais mais limitantes ao crescimento e à produção do algodoeiro (*Gossypium hirsutum* L.) em regiões tropicais, devido à exposição a dias nublados e com baixa disponibilidade de radiação. Neste estudo, o desenvolvimento inicial do algodoeiro foi avaliado em intervalos alternados de sombreamento de 2, 4 e 8 dias, sombreamento contínuo e ausência de sombreamento (controle), utilizando-se tela preta para redução de 50% da intensidade de luz. O sombreamento contínuo reduziu o comprimento total da raiz em 30% comparado ao controle. Reduções de comprimento foram verificadas nas raízes mais finas (0-0,5 mm), sendo aproximadamente de 47% nos tratamentos com sombra contínua e a cada 2 dias; 33,8% com intervalo de 8 dias; e 11% com intervalo de 4 dias. Plantas sombreadas continuamente e com intervalo de sombra de 2 dias apresentaram maior altura, porém menos folhas. O sombreamento a cada 4 dias ou contínuo diminuiu a biomassa vegetal e o acúmulo de carboidratos nas folhas e botões florais. Em conclusão, o sombreamento contínuo ou alternado a cada 4 dias reduz o teor de carboidratos nas folhas e hastes; o sombreamento contínuo reduz o comprimento total da raiz do algodão; e o comprimento da raiz das raízes mais finas (0-0,5 mm) é afetado pela sombra contínua ou alternada a cada 2 dias.

**Palavras-chave:** baixa radiação; massa de matéria seca reprodutiva; diâmetro radicular; comprimento radicular



## Introduction

Cotton (*Gossypium hirsutum* L.) is the most produced and consumed natural fiber in the world. More than 109 million tons of textile fibers were produced in 2020, and cotton accounted for 24.2%, synthetic fibers 62.5% and all other natural and manmade fibers 13%. In 2020, cotton represented 76% of all natural fibers produced for the textile industries (Textile Exchange, 2021). Despite being a hardy plant, high cotton yields depend on the appropriate combination of abiotic factors such as temperature, light, water and nutrients. Particularly in tropical regions and rainfed systems, rainy and cloudy days limit radiation availability, especially at the beginning of the plants development, which results in reduced yield (Echer & Rosolem, 2015) and root growth (Echer et al., 2019).

Cotton growth yields are impacted by genetic and environmental factors, such as climatic conditions, water availability, soil quality and management practices (Saranga et al., 2001; Liu et al., 2013). Cotton has been grown as a main crop in Western São Paulo during the rainy season so that recommended planting dates are between mid-October and late-January (Embrapa, 2019). As a consequence, the developing plant is subjected to an environment of low radiation supply due to high incidence of rain, limiting photosynthetic activity, yield and fiber quality (Luo et al., 2017).

Light is the predominant source of energy for photosynthesis and also an important signal for plant growth and development (Hu et al., 2012; Lee et al., 2017). Thus, shading can affect the morphophysiological responses of plants, since it acts directly on the photochemical and biochemical reactions of photosynthesis (Lisboa et al., 2019). The variation in photosynthetic activity due to shading in plants can cause irreversible damage such as decreased growth, hormonal imbalance, low carbohydrate accumulation, low root growth, fewer leaves, flowers and flower buds, and the main consequence of this is a yield and fiber quality decrease (Brand et al., 2016; Yang et al., 2017).

Ying et al. (2019) reported that shading inhibits terminal shoot growth and promotes dormancy of pink 'jambo' (*Syzygium samarangense*) shoots, as a consequence of the reduction of total levels of soluble sugar, sucrose, glucose, fructose, and starch in the leaves, reducing the accumulation of carbohydrates and vegetative growth. Echer et al. (2019) evaluated the root development of cotton cultivars subjected to shading at the initial stage of crop development and observed a decrease in shoot carbohydrate content, affecting shoot dry weight accumulation and root growth, regardless of the cultivar.

Shading effects on root growth are already known, but previous studies were carried out throughout the period, in extreme environmental conditions that hardly occur in field crops, since rains (cloudy conditions) are daily or weekly and alternate with sunny periods (Echer et al., 2019). However, alternating shading in short periods can mitigate the effects

of a longer period of shade on cotton root growth. Thus, the objective of this work was to evaluate the initial development of cotton under intermittent and continuous shading from emergence until flowering onset.

## Materials and Methods

Cotton plants were grown in a greenhouse, with a dimension of 10 × 20 m covered with plastic film with 90% solar transmissivity, municipality of Presidente Prudente, SP, Brazil (22° 06' 59" S, 51° 27' 02" W), between October and December 2017. The experimental design was a randomized block with four repetitions. Treatments were: unshaded, 2-day, 4-day, 8-day shade intervals and continuous shade. Shading intervals corresponded of 2, 4 or 8 intercalated days of shade and no shade.

Global solar radiation during the experiment is in Figure 1. Shading was imposed after plant emergence according to the treatment, i.e. 2-day - 2 days of shading and 2 days without shading.

Seeds (cultivar TMG 47B2RF) were placed on germination paper, moistened with distilled water, and sent to a germination chamber at 25 °C. After the appearance of the radicle (2 mm) (36 hours), two seedlings were selected and planted per rhizotron 0.30 m-width × 1.00 m-deep, made of PVC pipes coated with fiberglass, cut in half in the longitudinal direction with a flat face of transparent tempered glass to allow monitoring cotton root system growth, being positioned with a distance of 50 cm from each other. The rhizotron was filled with 60 kg of substrate, which had the following chemical characteristics: pH CaCl<sub>2</sub> = 7.0; organic matter = 60 g dm<sup>-3</sup>; P = 656.0 mg dm<sup>-3</sup>; S-SO<sub>4</sub><sup>2-</sup> = 348.0 mg dm<sup>-3</sup>; B = 0.9 mg dm<sup>-3</sup>; Zn = 16 mg dm<sup>-3</sup>; K = 16.0 mmol<sub>c</sub> dm<sup>-3</sup>; Ca = 218.0 mmol<sub>c</sub> dm<sup>-3</sup>; Mg = 49.0 mmol<sub>c</sub> dm<sup>-3</sup>; SB = 284.0 mmol<sub>c</sub> dm<sup>-3</sup>; CEC = 295.0 mmol<sub>c</sub> dm<sup>-3</sup>; and 97% base saturation. Fifteen days after emergence (DAE), thinning was carried out, leaving only one plant per

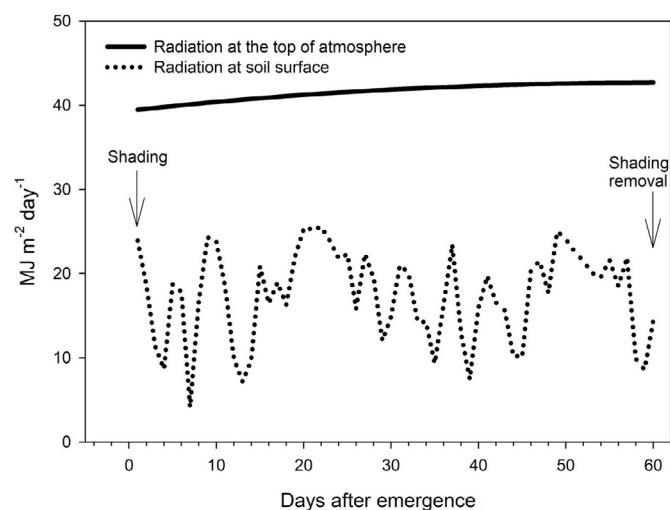


Figure 1. Solar radiation at the top of the atmosphere and soil surface during the experiment. Presidente Prudente, SP, Brazil.

rhizotron. Irrigation was carried out to replace the water lost by evapotranspiration, so that the soil moisture level was maintained at the field capacity, using on average 0.2 L d<sup>-1</sup> of water per rhizotron in the first 30 days and 0.4 L d<sup>-1</sup> from the first square stage until the end of the experiment.

A black shade cloth providing 50% reduction in light intensity and supported by a wire structure was used on shaded treatments, which was always 50 cm above the cotton plant. Blocks were 2 m apart from each other on East-West position, to avoid side to side shading. Accumulated radiation and the number of days with shading in each treatment is on [Table 1](#).

Root growth evaluation was performed at 8, 15, 23, 29, 37, and 43 DAE, according to [Tennant \(1975\)](#), using transparent film plastics over the glass and drawing the root system using a different colour pen for each evaluation. Plastic films were placed on a grid paper (0.5 × 0.5 cm) and the number of intersections of the main root and lateral roots were counted and used to estimate the root length, using the following formula:  $L = N \times (11/14)$ , where L is the root length and N the number of intersections. The total root length was obtained by the sum of tap root and lateral root lengths. Plant height and leaf number were evaluated at 8, 15, 23, 29, 37, and 43 DAE.

When the first-flower phenological stage ([Marur & Ruano, 2001](#)) was reached (60 DAE), the shoot was cut at soil surface and separated into leaves, stem, flower buds and flowers and oven dried at 65 °C for 48 hours, weighed for dry weight determination, and ground in a Wiley mill for later quantification of total soluble sugars, according to the methodology of [Dubois et al. \(1956\)](#), which consists of centrifuging the vegetable tissue with 80% ethanol and then

**Table 1.** Radiation accumulated and number of days with shading in each light restriction treatment.

Treatment	Accumulated radiation (MJ)	Days of shading
Unshaded	1052	-
2-d shade interval	778	30
4-d shade interval	769	32
8-d shade interval	758	32
Shaded	526	60

**Table 2.** Length, average diameter and length by diameter class of cotton roots according to the shading regime at 60 days after emergence.

Treatments	Length (m)	Average diameter (mm)	Length (m)				
			Diameter class (mm)				
			0-0.5	0.5-1.0	1.0-2.0	2.0-3.0	>3.0
Unshaded	1013 a	0.25 ab	687 a	218	85	15	7
2-d shade interval	850 ab	0.20 c	365 c	193	113	24	13
4-d shade interval	979 a	0.26 b	613 ab	236	99	19	11
8-d shade interval	956 a	0.23 ab	459 abc	205	103	21	12
Shaded	703 b	0.36 a	373 c	194	100	21	15
CV% <sup>a</sup>	13.5	13.8	31.9	29.8	27.8	60.0	89.0
HSD <sup>b</sup>	187.0	0.05	245.0	96.0	58.0	18.0	16.0

Averages followed by the same letter on the column do not differ from each other by the HSD Tukey test at the 0.05 probability level; <sup>a</sup>CV - Coefficient of variation; <sup>b</sup>HSD - Honestly significant difference.

using the supernatant to quantify sugars with 5% phenol and concentrated sulfuric acid.

The cotton root system was extracted from the substrate by removing the glass plate and using a fine jet of water. Roots were cut into pieces of approximately 1.5 cm to spread in the scanner tray and avoid root overlapping. Then, the samples were washed and placed in 0.5 L pots with 50% alcohol and stored in a refrigerator (~3 °C). Total root length, average root diameter and the length per class of diameter were evaluated using scanner (Epson XL 10000 - Japan) and WinRizo Pro 2017 software (Regent Instruments Inc. - Canada) for measurements.

Subsequently, roots samples were oven dried and analyzed for carbohydrate content ([Dubois et al., 1956](#)). Carbohydrate accumulation in shoot and roots was calculated as a product of the dry matter content and mass.

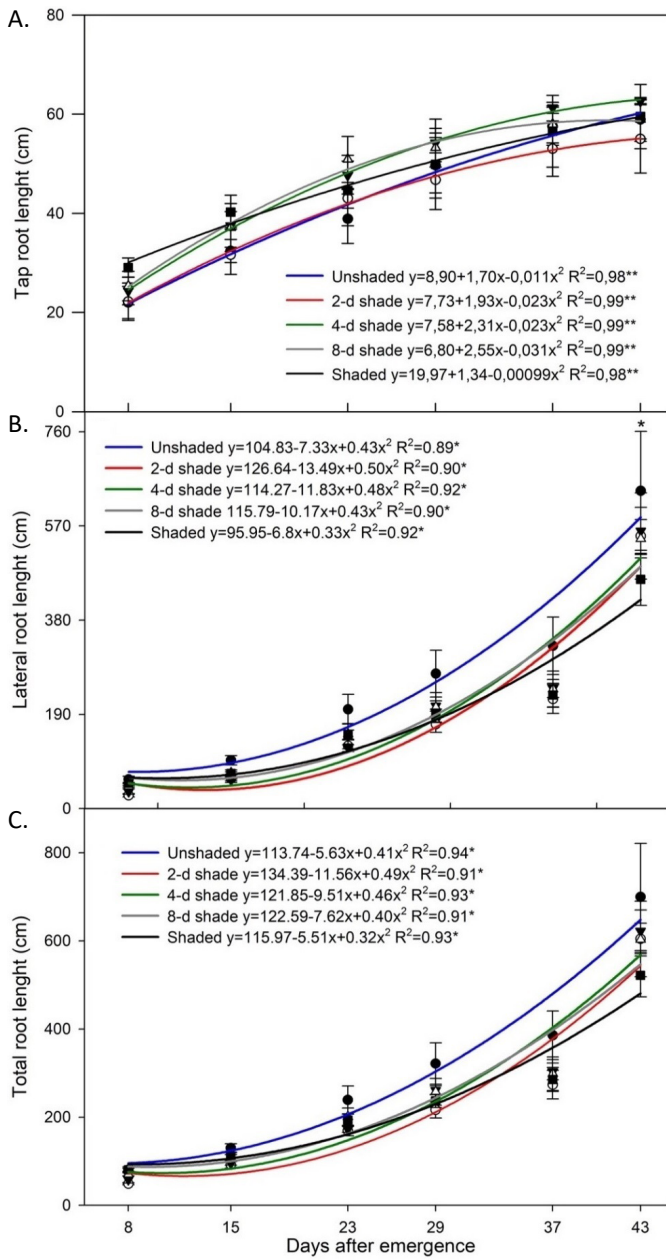
Total, tap and lateral root growth, number of leaves and plant height were submitted to regression analysis as a function of DAE. The other parameters were subjected to analysis of variance (F-test) and the means were compared by the Tukey honestly significant difference (HSD) test, at the level of 5% probability.

## Results

Two-day intermittent shading or continuous shade reduced the total root length by 30%, respectively, compared to unshaded. Reduction in length by 45.7% (continuous) and 46.8% (2-d) occurred in the thinner roots (0-0.5 mm). In addition, the continuous shading and the 2-d shading interval showed the largest and smallest mean root diameter, respectively ([Table 2](#)).

There was no significant difference among shade treatments on tap root length up to 43 days after emergence ([Figure 2A](#)) and total root length ([Figure 2C](#)), however the continuous shade decreased lateral root length compared to unshaded treatment at 43 DAE ([Figure 2B](#)).

The height of plants was measured at 8, 15, 23, 29, 37, and 43 DAE with a ruler, and on the same occasion the leaf count of each treatment was performed, and plant height was higher in the shaded treatment compared to unshaded treatment after 37 DAE ([Figure 3A](#)). However, the continuously shaded



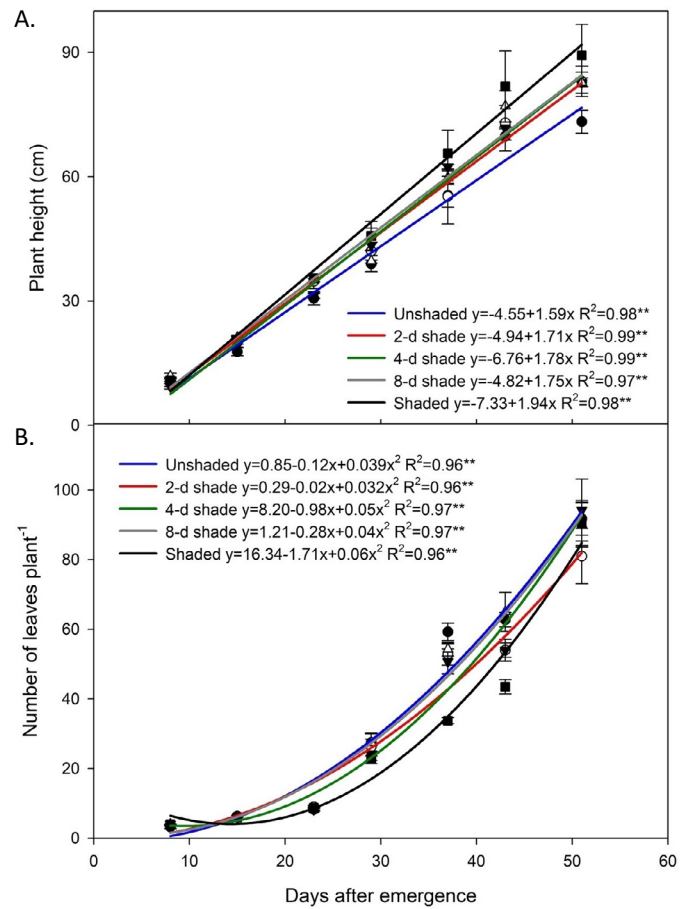
Vertical bars show the standard error of average. \* Significant at the 0.05 probability level. \*\* Significant at the 0.01 probability level.

**Figure 2.** Growth of the tap root (A), lateral roots (B), and total root (C) cotton growth submitted to different intermittent shade regimes.

**Table 3.** Content and accumulation of carbohydrates in leaves, roots, stem and squares of cotton submitted to different shading regimes.

Treatments	Carbohydrate content (mg g <sup>-1</sup> DW)				Carbohydrate accumulation (mg plant <sup>-1</sup> )			
	Leaf	Root	Stem	Square	Leaf	Root	Stem	Square
Unshaded	14.6 a	4.1	19.5 a	17.3	1185 a	45.2	250.7	211 a
2-d shade interval	14.8 a	3.0	19.4 a	15.6	734 b	46.4	176.4	142 ab
4-d shade interval	8.9 b	2.7	13.4 b	14.5	711 b	61.0	235.4	120 b
8-d shade interval	15.0 a	3.5	17.7 a	16.1	798 ab	51.6	214.3	110 b
Shaded	13.2 a	3.2	12.0 b	14.5	503 b	48.5	182.0	98 b
CV% <sup>a</sup>	11.1	27.8	10.9	12.6	22.7	41.6	38.2	23.3
HSD <sup>b</sup>	3.3	2.1	4.0	4.4	404.1	47.4	182.5	71.8

Averages followed by the same letter on the column do not differ from each other by the HSD Tukey test at the 0.05 probability level; <sup>a</sup>CV - Coefficient of variation; <sup>b</sup>HSD - Honestly significant difference.



Vertical bars show the standard error of average. \*\* Significant at the 0.01 probability level.

**Figure 3.** Plant height (A) and number of leaves per plant (B) as affected by intermittent shade regime.

plants or in the 2-d interval showed a lower number of leaves per plant (Figure 3B).

Leaf carbohydrate content was lower in plants under a 4-d shade interval; on the stem, it was lower on shaded and 4-d shade interval (Table 3). In addition, there was no effect of shading on the carbohydrate content on roots and squares. Also, carbohydrate accumulation was reduced by continuously shaded, 2-d and 4-d shading intervals on leaves, whereas only the 2-d interval reduced the accumulation on squares compared to the unshaded condition (Table 3), with no effects observed on stem and roots.

**Table 4.** Number of leaves, squares, and dry weight according to the shading regime.

Treatments	Leaves	Squares	Dry weight (g plant <sup>-1</sup> )				Dry weight (g un <sup>-1</sup> )		
	plant <sup>-1</sup>		Leaf	Stem	Square	Root	Total	Square	Leaf
Unshaded	101 a	42 a	86 a	69	11 a	4.9 a	170 a	0.27	0.84 a
2-d shade interval	87ab	38 ab	62 b	65	9 ab	3.5 ab	140 ab	0.26	0.71 ab
4-d shade interval	91 ab	33 ab	51 b	60	7 bc	2.0 b	120 bc	0.22	0.56 b
8-d shade interval	96 a	28 ab	64 b	70	8 bc	2.6 ab	144 ab	0.30	0.67 ab
Shaded	71 b	21 b	36 c	54	6 c	2.7 ab	99 c	0.30	0.51 b
CV% <sup>a</sup>	10	24	15	16.5	14	31.5	11.3	22.7	14.1
HSD <sup>b</sup>	21.7	17.7	21.0	23.7	2.7	2.2	34.5	0.13	0.2

Averages followed by the same letter on the column do not differ from each other by the HSD Tukey test at the 0.05 probability level; <sup>a</sup>CV - Coefficient of variation; <sup>b</sup>HSD - Honestly significant difference.

Continuously shaded plants produced fewer leaves structures than those submitted to the 8-d interval and unshading conditions, while the number of squares did not differ from those from 2-d, 4-d, and 8-d plants (Table 4). In addition, there was a reduction on leaf dry weight in all shaded treatments; squares on 4-d and 8-d shading intervals; roots on 4-d shade interval and total dry weight on 4-d shading interval and continuous shade treatments. There was no effect of shading on single square dry weight, but 4-d or continuous shading reduced leaf dry weight (Table 4).

## Discussion

Shading decreased cotton root system growth, and the reduction occurred in the finer roots, between 0 and 0.5 mm in diameter (Table 1). Shaded treatments decreased the dry matter mass in maize (Xue et al., 2016) and rice (Wu et al., 2017). However, unshaded treatment (Figure 2C), showed higher total root growth, a response that was already expected, because when the plant has no light restriction, its photosynthesis is not altered as the carbohydrates production, showing that cotton root development is impaired at the beginning of the plants cycle when subjected to low sunlight incidence.

Unshaded cotton showed a lower final plant height (Figure 3A), indicating a greater etiolation on shaded treatments. This is due to the negative hormonal imbalance, such as decreased auxin production. Wu et al. (2017) and Li et al. (2019) also found negative effects of shading on plant growth, such as less vegetative branches. Shaded resulted in lower number of leaves, and this behavior can be explained due to the photosynthetic imbalance and consequent decrease in the accumulation of carbohydrates caused by the long period of shading. Studies show that low solar radiation significantly decreases photosynthetically active radiation (PAR) in plants (Huber et al., 2014; Wu et al., 2017). However, shading results in thinner stems with elongation and reduced mechanical stability of the entire plant (Huber et al., 2014).

Carbohydrates are the main source of energy for plant development, and production source comes from photosynthesis, through light energy, so any light stress can directly affect crop development. Four-day shade intervals resulted in a lower carbohydrate content in the leaves and in the stem, which contributed to reducing the accumulation of

leaf dry matter, squares and lower total biomass production in cotton, a fact also found by Chen et al. (2017), who observed that shading decreased the cotton yield and fiber quality, factors that are directly linked to changes in photosynthesis and carbohydrate concentrations during its development. Confirming this energy imbalance in the plant, the unshaded treatment showed a higher carbohydrate content and accumulation in all parts of the plant, thus concluding that shading is an abiotic factor that acts negatively at the initial stage of cotton development, since luminosity is a factor that acts directly in the photosynthetic process (Lisboa et al., 2019).

The lower content and accumulation of carbohydrates in shaded treatment resulted in fewer leaves and squares, a smaller plant height and the main consequence would be the decrease in crop yield. Environmental stressors such as low temperatures and light deficiency are restrictive to cotton production (Lv et al., 2013; Zhao et al., 2018). Previous studies have shown that shading significantly increased fiber sucrose content but decreased the rate of sucrose transformation and cellulose content, which consequently reduced fiber yield and quality due to carbohydrate deficiency (Wu et al., 2017; Zhao et al., 2018; Lisboa et al., 2019), since the fiber is composed of 95% cellulose.

Light intensity and quality are among the most critical environmental factors for cotton physiology and biochemistry (Yang et al., 2018). For most cultivated plants, even an increase or decrease in light intensity leads to considerable changes in leaf morphology and structure (Wu et al., 2017). According to previous studies, dry weight of roots, stems, leaves, and whole plants, as well as the photosynthetic rate, transpiration, and stomatal conductance, and the stem diameter decreased in low light conditions (Yang et al., 2014; Echer et al., 2019). In low light conditions, cultivated plants produce thinner leaves compared to the leaves of plants grown in full light conditions (Wu et al., 2017). However, shading environments have increased plant height and lodging rate, which makes it difficult to transport nutrients, water, and photosynthetic products and, finally, causes huge losses to agricultural production. Su et al. (2014) observed that shading of soybeans caused by the corn consortium reduced the photosynthetic rate, the leaf area, and biomass accumulation of soybean cultivars, including stem, leaves and roots and finally on yield.

A direct relation between carbohydrate inputs in leaves and squares and the measured dry weight is expected (Echer et al., 2019). According to the results among the tested shade treatments, the 4-day interval seemed to be the most responsive for growth reduction when compared to unshaded plants, given the decrease in carbohydrate content (leaf and squares) and accumulation (squares), and the lower root, leaf, square, and total dry weight (Tables 3 and 4). This interval would be long enough to reduce the carbohydrate production by leaves, but, at the same time, insufficient for physiological recovery in the post-shading phase before resumption of shading. For the 2-d procedure, the intervals would be too short, and the plants would already have unrestricted light within a short period, although thinner roots were noted (Table 2). For the 8-day interval, the plants would remain unshaded for a longer time after shading, thus enabling a compensatory effect. When comparing carbohydrate accumulation values, unshaded and 8-d leaves do not differ from each other; and unshaded and 2-d squares also do not differ from each other (Table 3). These responses indicate that short shading intervals induce more immediate effects that are observed only in leaves. Thus, a reduction in accumulation in reproductive structures would require a longer shading period, which indeed occurred at both 4-d and 8-d intervals. Therefore, for shorter and more frequent periods, an effect is more likely to occur on carbohydrate production by leaves, while for longer and less frequent periods on carbohydrate mobilization to reproductive sinks.

Shading reduces the total root length (continuous shade) and the root length of fine roots (continuous shade and 2-d interval), but the mean root diameter decreases (2-d) and increases (4-d) with shading. Carbohydrate content of leaves and stem is decreased by continuous shade or 4-d intervals and carbohydrates accumulation on leaves and squares when occurs constantly or at 4 and 8-day intervals. Total biomass accumulation is decreased by shading or 4-d shade intervals, but the reduction in reproductive biomass occurs only under continuous shade.

## Conclusion

Short period of alternated shading (2-days) is more injurious to early cotton root growth, but medium period (4-days) impairs leaf carbohydrate content resulting in less leaf, square and root biomass.

The main effect of continuous shading was the reduction of total and the finest roots length and the average root diameter increase while the 8-day shade interval resulted in a decrease of leaf and square biomass.

Future investigations should focus on strategies to lessen the effect of shading at early stages since it impairs root and shoot growth in cotton plants.

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## Compliance with Ethical Standards

**Author contributions:** Conceptualization: FRE; Data curation: FAM, PHG, JP; Formal analysis: FAM, PHG, JP; Investigation: FAM, PHG, JP; Methodology: FRE; Validation: FRE; Writing original draft: FAM, PHG, JP; Writing review & editing: FRE.

**Conflict of interest:** The authors declare that there is no conflict of interest.

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