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Crop Science/ Original Article

Morphogenetic, physiological, and productive of forage peanut responses to shading

Abstract – The objective of this work was to evaluate the morphogenetic, structural, physiological, and productive traits of forage peanut (*Arachis pintoi*) subjected to different levels of artificial shading in the field. The 'Amarillo MG-100' forage peanut was planted in April 2015, and the evaluations were carried out from May 2017 to April 2018. The treatments were: 0, 30, 45, and 75% of artificial shading. There was no significant effect of shading on the morphogenetic traits of forage peanut. Shading increased final leaf length and canopy height and decreased the leaf area index and number of stolons. Photosynthesis, transpiration rate, stomatal conductance, chlorophyll b, and leaf temperature increased quadratically as a function of shading. There was a linear positive effect on the growing cycle length and a quadratic effect on the production of dry matter mass, with the maximum at 30% shade. Forage peanut harvested at 95% light interception (11-cm height) shows adaptation to shading up to 45%, with increased leaf size, canopy height, photosynthetic rate, stomatal conductance, transpiration rate, and chlorophyll b. In addition, plants at 30% shading show a higher yield than those growing under full sun.

Index terms: *Arachis pintoi*, leaf measurements, light interception, net photosynthesis.

Respostas morfogenéticas, fisiológicas e produtivas de amendoim-forrageiro ao sombreamento

Resumo – O objetivo deste trabalho foi avaliar as características morfogenéticas, estruturais, fisiológicas e produtivas do amendoim-forrageiro submetido a diferentes níveis de sombreamento artificial no campo. O amendoim-forrageiro 'Amarillo MG-100' foi plantado em abril de 2015, e as avaliações foram realizadas de maio de 2017 a abril de 2018. Os tratamentos foram: 0, 30, 45 e 75% de sombreamento artificial. Não houve efeito significativo do sombreamento sobre as características morfogenéticas do amendoim-forrageiro. O sombreamento aumentou o comprimento final da folha e a altura do dossel e diminuiu o índice de área foliar e o número de estolões. A fotossíntese, a taxa de transpiração, a condutância estomática, a clorofila b e a temperatura das folhas aumentaram quadraticamente em função do sombreamento. Houve efeito linear positivo sobre o tamanho do ciclo de crescimento e efeito quadrático sobre a produção de massa de matéria seca, com o máximo a 30% de sombra. O amendoim-forrageiro colhido a 95% de interceptação luminosa (11 cm de altura) apresenta adaptação ao sombreamento de até 45%, com aumento do tamanho das folhas, da altura do dossel, da taxa fotossintética, da condutância estomática, da taxa de transpiração e da clorofila b. Além disso, as plantas sob sombreamento de 30% apresentam maior rendimento do que aquelas que crescem sob a luz do sol.

Termos para indexação: *Arachis pintoi*, medidas foliares, interceptação luminosa, fotossíntese líquida.

Introduction

Agroforestry systems are interesting options for preserving the environment and increase food production. However, the achieving of sustainability and intensification of integrated systems depends on the proper choice of the species that will constitute it. Warm-season forage legumes, as an understory cover of agroforestry systems, enhance the forage quality and soil fertility in comparison with grasses.

Forage peanut (*Arachis pintoi* Krapov. & W.C. Greg.), a stoloniferous legume, is notable for its adaptation to shaded environments with higher-herbage yield and nutritive value than other warm-season forage legumes (Andrade et al., 2004; Lista et al., 2019). Generally, shaded forage legumes show decreased net photosynthesis, stomatal conductance, and transpiration rate (Baligar et al., 2010; Guenni et al., 2018). However, forage peanut has a different physiological response to moderate shading, increasing its stomatal conductance and transpiration and maintaining the photosynthetic rate (Baligar et al., 2010).

In addition, except for the work by Gobbi et al. (2009), studies on shaded forage peanut have been carried out using a pre-defined regrowth period, or targets for canopy height developed under full sunlight conditions (Andrade et al., 2004; Baligar et al., 2010; Freitas et al., 2016; Yemataw et al., 2018; Lista et al., 2019); these grazing management strategies may not be suitable for use under shade.

Forage peanut growing in full sunlight has a regrowth pattern similar to those of tropical grasses and shows a strong correlation among 95% of canopy light interception, maximum forage accumulation, and increased animal performance (Brunetti et al., 2016; Kröning et al., 2019). However, this grazing management under shade should be better understood, since grazing management and shading directly affect the structural, physiological, and productive characteristics of tropical forage legume (Baligar et al., 2010; Guenni et al., 2018).

Based on the aforementioned arguments, the hypothesis is that, when managed with intermittent defoliation at 95% light interception, forage peanut under shade conditions should be able to adjust its growing pattern to maintain the pasture yield.

The objective of this work was to evaluate the morphogenetic, structural, physiological, and productive traits of forage peanut subjected to different levels of artificial shading in the field.

Materials and Methods

Forage peanut 'Amarillo MG-100' was planted in April 2015, using stolons from plants belonging to the agrostological field of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). The evaluations were carried out from May 2017 to April 2018, at the experimental field of the Animal Science Department of UFVJM, in the municipality of Diamantina, in the state of Minas Gerais, Brazil (18°12'S, 43°30'W, at 1,387 m altitude). The climate of the region is Cwb, according to the Köppen-Geiger's classification, which is characterized by an altitude tropical climate with rainy summer and dry winter. Meteorological data were obtained from a station of the Instituto Nacional de Meteorologia (Inmet), located approximately 10 km from the experimental area in Diamantina. Rainfall distribution, temperature, and air relative humidity variation along the experimental period is reported in Figure 1.

The soil at the experimental area was classified as Neossolo Quartzarênico órtico latossólico, according to Santos et al. (2018), i.e., Arenosols or Quartzipsamments, with texture characterized by 779 g kg^{-1} sand, 110 g kg^{-1} clay, and 111 g kg^{-1} silt. The soil showed the following chemical characteristics at a 0–20 cm depth: 5.5 pH (H_2O); 0.9 mg dm^{-3} P; 19.0 mg dm^{-3} K; 0.9 cmol$_c$ dm^{-3} Ca^{+2}; 0.4 cmol$_c$ dm^{-3} Mg^{+2}; 0.0 cmol$_c$ dm^{-3} Al^{+3}; 3.33 cmol$_c$ dm^{-3} CEC; 1.35 cmol$_c$ dm^{-3} sum of

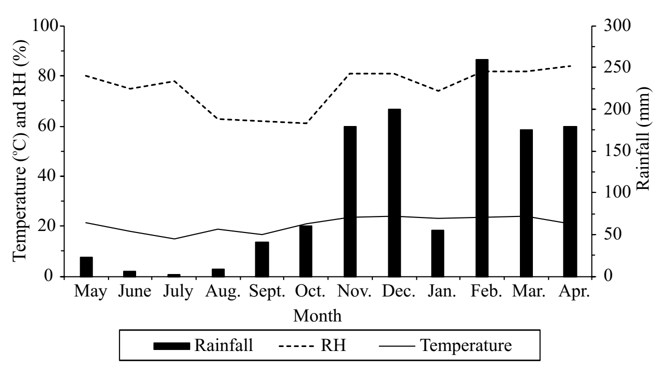

Figure 1. Monthly temperature, air relative humidity (RH), and rainfall, in the municipality of Diamantina, in the state of Minas Gerais, Brazil during the experimental period from May 2017 to April 2018. Source: Inmet (2020).

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bases (SB); 1.98 $cmol_c$ dm^{-3} H+Al; 1.86 dag kg^{-1} organic matter; and 28.4 mg dm^{-3} P-rem.

The fertilization recommendations by Ribeiro et al. (1999) for warm-season legumes were carried out, using 90 kg ha^{-1} phosphorus pentoxide (P_2O_5) as single superphosphate fertilizer, and 1,000 kg ha^{-1} dolomitic limestone. The maintenance fertilizers were applied at 35 days after planting and at the beginning of each season, as follows: 40 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} K_2O in the form single superphosphate and potassium chloride, respectively, diluted in water and distributed with watering cans.

Shading was implemented one day after planting, by using a wooden frame and black polypropylene screens with different degrees of radiation transmission, without changing the light quality. The screens covered the entire experimental unit, including the sides, at 2.0 m height above ground. The experimental design was completely randomized, with four treatments of 0, 30, 45, and 75% artificial shading, with six replicates. The experimental units had 2.5 m spacing between them, and each one had 0.5 m alley between rows and 5.0 m^2 area.

Photosynthetically active radiation (PAR), shade projection, and leaf area index (LAI) were quantified weekly, throughout the day, at 9:00, 12:00, and 15:00 h, by using a ceptometer (AccuPAR LP 80, Decagon Devices, Pullman, WA, USA). A Field Scout red to far red meter (Spectrum Technologies, Aurora, IL, USA) was used to measure red-to-far-red light ratio (R: FR). Temperature was monitored daily by using a chapel-type thermometer (TM-38 CAP, Equitherm, Curitiba, PR, Brazil) located in the center area of each treatment.

Since the establishment in April 2015, plants were cut at half height when their canopy reached 95% light interception (LI), to not impact herbage accumulation (Alonzo et al., 2017; Sbrissia et al., 2018). At the beginning of the experimental period in May 2017, nine stolons were marked per experimental unit for evaluations of morphogenic and structural characteristics. Stolons were evaluated twice a week, until reaching 95% LI in each season of the year. The following characteristics were measured: leaf appearance rate (Bircham & Hodgson, 1983); stolon elongation rate; petiole elongation rate; leaf elongation rate (Skinner & Nelson, 1995) measured in length and width; number of live leaves; final leaf and petiole length; canopy height (from the soil surface to the final leaf); and leaf lifespan.

In each season of the year, when the canopy reached 95% LI, chlorophyll content and photosynthesis were measured. The contents of chlorophyll a and b (Chl a, Chl b) were measured in the median region of the youngest fully expanded leaf, by using a chlorophyll meter (ClorofiLOG CFL 1030, Falker, Porto Alegre, RS, Brazil). Photosynthesis [leaf photosynthesis (A), leaf transpiration rate (E), stomatal conductance (Gs), water-use efficiency (WUE) obtained by the ratio A/E, and leaf temperature] was measured in the same leaves, using a portable photosynthesis analysis system (LCpro-SD, ADC BioScientific, Hoddesdon, UK), with the light intensity in the chamber at 1,200 mmol photons m^{-2} s^{-1} photosynthetic photon flux density. These readings were carried out on sunny days, between 8:00 and 10:00 h on six leaves per experimental unit.

After the physiological evaluations, three samples per experimental unit were harvested and cut at half height. The measurements were made using 0.25 m^2 (0.5 × 0.5 m) quadrats that were randomly thrown three times in each experimental unit. The entire unit was mowed at half height after each evaluation. After harvesting, the morphological separation of the fresh forage was performed. The samples were separated into stems and leaves and dried in a forced-air oven at 55°C, until they reached a constant weight.

All data were subjected to the analysis of variance, by considering the shading levels as fixed effects and the experimental error as a random effect. Whenever the analysis indicated that there was a significant effect for the shading level, the averages were compared, using linear and quadratic orthogonal contrasts, at 5% probability. The variables were analyzed using the statistical software R (R Core Team, 2020).

Results and Discussion

The black polypropylene screens linearly reduced the incident PAR, increasing the shade projection; however, shade has not affected the ratio of red to far-red light and temperature inside the experimental unit. The screens provided 1.11 average ratio of red to far-red and 20.7°C temperature (Table 1). Shading intensity provided by black polypropylene screens depends on the difference of hole sizes of the screens.

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This allows of even the highest-shade level to receive all wavelengths from sunlight, affecting only radiation quantity and not quality. However, artificial shading through screens can be used to investigate plant physiological responses and provides a more uniform light regime than that found in the forest understory (Sevillano et al., 2018).

There was no significant effect of shading on the morphogenetic traits of forage peanut (Table 2). Artificial shading, without changing light quality, does not seem to be a good option for the morphogenesis evaluation, since the effects of shading on the petiole and stolon elongation rate are mainly due to the change of light quality (Héraut-Bron et al., 2001).

No significant effect of shading was observed on the number of live leaves (NLL) and leaf lifespan (LL). However, shading increased the final leaf length (FLL) and canopy height (CH), and decreased the leaf area index (LAI) and number of stolons (NS). Larger leaves and taller canopies are responses to shading in an attempt to increase the incident light uptake by plants. The etiolation is a common plant response to shading that increases the plant capacity to capture light by lifting its leaves (Gobbi et al., 2009; Paciullo et al., 2017). In addition, shading reduces the stimulus for the emergence of new stolons and this is possibly the main factor that decreases LAI (Héraut-Bron et al., 2001; Gobbi et al., 2009).

As to the physiological traits, there was effect of shading on photosynthesis, transpiration rate, stomatal conductance, chlorophyll b, and leaf temperature. All these variables increased quadratically as a function of shading (Table 2).

Shading intensity up to 45% favored an increase of the stomatal conductance and transpiration rate, probably due to improvements of the environmental conditions, mainly soil moisture (Monteiro et al., 2016; Nascimento et al., 2019), although this condition was not evaluated in the present study. This response pattern increased the internal CO_2 concentration which, when associated with increased chlorophyll b indices, results in high-photosynthesis rate. However, the transpiration rate directly affects the WUE because it increases the quantity of transpired water per amount of fixed CO_2, decreasing the efficiency of carbon fixation. The lack of effect on WUE was due to the increase of transpiration and photosynthesis rates. In addition, Gobbi et al. (2011) observed some morphoanatomical adaptations of forage peanut, such as a higher proportion of lacunar parenchyma and vascular bundle sheath cells in leaves, which may have contributed to improve the physiological traits. It is worth mentioning that, in the long term, this photosynthesis increasing can be especially important because it favors the plant's reserves, especially in the roots, which will favor the system perenniality. However, this should be evaluated in future research.

Leaf temperature is closely associated with leaves-water loss (Monteiro et al., 2016). In the present study, despite the increase of stomatal conductance and transpiration rate, the shading probably decreased the evapotranspiration of the system (Nascimento et al., 2019), affecting the leaf boundary layer resistance, hindering the water loss and heat dissipation and, consequently, increasing the leaf temperature.

There was no effect of shading on leaf, stem, and dead material percentage of forage peanut (Table 2). Nonetheless, there was a significant effect of shading on the growing cycle length (GCL) and dry mass production (DMP). The growing cycle length (GCL)

Table 1. Incidence of photosynthetically active radiation (PAR), ratio of red to far-red light (R:FR), shade projection, and temperature, for each treatment of forage peanut (*Arachis pintoi*) 'Amarillo MG-100', from May 2017 to April 2018, in the municipality of Diamantina, in the state of Minas Gerais, Brazil.

Trait	Artificial shading(1) (%)				SEM	p-value(2)	
	0	30	45	75		Linear effect	Quadratic effect
PAR (μmol m^{-2} s^{-1})	501	321	262	134	32.1	***	ns
Shade projection (%)	-	29.6	45.3	73.0	8.54	***	ns
R:FR ratio	1.09	1.11	1.18	1.07	1.97	ns	ns
Temperature (°C)	21.0	20.4	20.8	20.5	2.37	ns	ns

(1)Shading was implemented using a wooden frame and black polypropylene screens with different degrees of radiation transmission. (2)Effects linear and quadratic due to shading level: ***, **, *significant at 0.1, 1, and 5% probability, respectively; and nsnonsignificant. SEM, standard error of the mean.

increased linearly because of shading. Intense shading (75%) resulted in five harvests in the year, while other treatments resulted in six harvests. Longer GCL is due to a lower number of stolons which decreases the ground cover, requiring taller canopies to reach the grazing management goal of 95% LI and, therefore, longer rest period and lower-annual yield.

Forage peanut showed a quadratic pattern for DMP, with the maximum at 30% shading, and maintained at 45% shade at least (Table 2). Highest DMP at 30% shading (44% higher than full sun) was mainly a result of increased photosynthesis rates (73% greater than full sun), a response ensured by increases of transpiration rate (43% greater than full sun), stomatal conductance (54% greater than full sun) and chlorophyll b indices (6.4% greater than full sun). In addition, shading of 45% resulted in a production similar to that under full sun, however with increases also of photosynthesis, transpiration, stomatal conductance, and chlorophyll b indices, of 51, 67, 85, and 15%, respectively. Almeida et al. (2019) working with *Calopogonium mucunoides, Pueraria phaseoloides, Macrotyloma axillare*, and *Neonotonia wightii* also observed an increase of DMP with shading, with maximum values between 30 and 45% of shade.

Table 2. Morphogenetic, structural, and physiological traits, and herbage mass for swards of forage peanut (*Arachis pintoi*) 'Amarillo MG-100' grown at artificial shade intensities in the field, from May 2017 to April 2018, in the municipality of Diamantina, in the state of Minas Gerais, Brazil.

Trait	Artificial shading(1) (%)				SEM	p-value(2)	
	0	30	45	75		Linear	Quadratic
Morphogenetic							
Leaf appearance rate (LAR, leaf per day)	0.05	0.06	0.06	0.07	0.02	ns	ns
Leaf elongation rate (LER, cm^2 per day)	0.03	0.04	0.04	0.05	0.02	ns	ns
Petiole elongation rate (PER, cm per day)	0.07	0.08	0.11	0.10	0.04	ns	ns
Stolon elongation rate (SER, cm per day)	0.05	0.05	0.05	0.19	0.15	ns	ns
Structural							
NLL(3) (leaves per stolon)	7.50	7.25	8.00	8.57	1.48	ns	ns
Final leaf length (FLL, cm)	1.78	2.06	2.23	2.28	0.22	***	ns
Leaf lifespan (LL, day)	41.0	44.6	46.4	56.1	15.4	ns	ns
Leaf area index (LAI)	4.13	3.68	3.32	3.14	0.22	**	ns
Canopy height (CH, cm)	9.60	10.4	11.2	13.5	2.64	***	ns
Number of stolons (NS)	2,527	1,446	1,352	1,035	553	***	ns
Physiological(4)							
A (μmol CO_2 m^{-2} s^{-1})	6.95	12.0	10.5	9.79	4.86	ns	***
E (mol H_2O m^{-2} s^{-1})	1.50	2.15	2.51	2.00	0.41	ns	***
Gs (μmol CO_2 m^{-2} s^{-1})	0.13	0.20	0.24	0.18	0.06	ns	***
WUE (μmol CO_2 mol^{-1} H_2O m^{-2} s^{-1})	5.33	6.00	4.33	3.94	1.95	ns	ns
Leaf temperature (°C)	24.2	25.3	25.4	25.4	0.32	ns	**
Chl a	28.1	28.3	28.8	27.9	2.66	ns	ns
Chl b	7.86	8.36	9.05	8.94	2.37	ns	*
Herbage mass(5)							
Leaf (percent of dry weight)	87.2	89.8	90.5	89.1	6.42	ns	ns
Stem (percent of dry weight)	11.4	8.80	9.02	10.8	5.69	ns	ns
Dead material (percent of dry weight)	1.34	1.42	0.48	0.01	2.06	ns	ns
Growing cycle length (GCL, day)	72.4	84.4	87.5	101	24.2	***	ns
DMP (kg ha^{-1} per year)	6,605	9,507	6,618	3,681	493	ns	*

(1)Shading was implemented using a wooden frame and polypropylene screens with different degrees of radiation transmission. (2)Effects linear and quadratic due to shading level: ***, **, *Significant at 0.1, 1, and 5% probability, respectively; and nsnonsignificant. SEM, standard error of the mean. (3)NLL, number of live leaves. (4)A, photosynthesis (μmol CO_2 m^{-2} s^{-1}); E, transpiration rate (mol H_2O m^{-2} s^{-1}); Gs, stomatal conductance (μmol CO_2 m^{-2} s^{-1}); WUE, water-use efficiency (μmol CO_2 mol^{-1} H_2O m^{-2} s^{-1}), obtained by the ratio A/E; LT, leaf temperature (°C); Chl a, chlorophyll a (Falker chlorophyll indices); Chl b, chlorophyll b (Falker chlorophyll indices). (5)DMP, dry matter mass production.

Physiological traits also increased at intense shading (75%); however, not enough to guarantee a DMP similar to that under full sun (Table 2). At intense shading, the number of stolons has reduced (144% lower than that under full sun) and, with that, longer rest period (39% greater than full sun) were necessary, reducing DMP by 79% in comparison with that under full sun. The physiological adjustments were not enough to compensate for the intense shade.

These response pattern to shading for physiological traits shows that forage peanut has potential even under intense shading and that it can be used as ground cover of understories, provided that the radiation reduction is not too severe.

Despite some structural differences, the canopy morphological composition of forage peanut remained practically constant with increasing shading. Other studies reported that forage peanut yield decreased under intense shading and that no changes were observed for the percentage of morphological components (Gobbi et al., 2009).

According to Silva et al. (2015), abiotic factors such as light and temperature affect the morphogenetic processes of forage plants, so that all processes involved in plant growth and development are reduced when plants are deprived of these factors. However, Johnson et al. (1994) working with *Arachis glabrata* observed leaf water and turgor potentials greater under 22% of shade than under full sun that resulted in greater forage accumulation.

Conclusion

Forage peanut (*Arachis pintoi)* 'Amarillo MG-100' harvested at 95% canopy light interception shows adaptation to shading up to 45%, by increasing leaf size, canopy height, photosynthetic rate, stomatal conductance, transpiration rate, and chlorophyll b; in addition, at 30% shading, forage peanut shows higher yield than plants growing under full sunlight.

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