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Morphogenesis and structure of Tifton 85 cultivated in subtropical climate and fertilized with nitrogen

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ABSTRACT. The experiment evaluated the morphogenesis and structure of Tifton 85 (*Cynodon spp.*) cultivated in subtropical climate and fertilized with nitrogen (N). The experiment was a completely randomized design with four levels of N (Zero; 75; 150 or 225 kg ha^{-1}) in nine replicates per area. The experimental animals were Suffolk female lambs. The grazing method was continuous to maintain the sward height at 15 cm ± 10%. The stem expansion increased by 0.000027 cm degree-days^{-1} to each kg N applied (linear model). According to nonlinear model, the highest stem expansion (0.0226 cm degree-days^{-1}) was observed with the use 220.1 kg ha^{-1} N. The leaf lifespan fitted a linear regression model, with increase of 50 degree-days leaf^{-1}, comparing the levels zero and 225 kg ha^{-1} of N. According to nonlinear regression model, the longest leaf lifespan (407.1 degree-days) was observed with the use 208.8 kg ha^{-1} N. With the maintenance of sward height at approximately 15 cm, we recommended to use 200 kg ha^{-1} N in Tifton 85 cultivated in subtropical climate.

Keywords: *Cynodon* spp., phyllochron, marked tillers, urea.

Morfogênese e estrutura do Tifton 85 cultivado em clima subtropical e adubado com nitrogênio

RESUMO. Objetivou-se estudar a morfogênese e a estrutura do Tifton 85 (*Cynodon spp.*) cultivado em clima subtropical e adubado com nitrogênio (N). O delineamento experimental foi o inteiramente casualizado com quatro níveis de N (Zero; 75; 150 e 225 kg ha^{-1}) em nove repetições por área. Os animais experimentais foram borregas das raças Suffolk. O método de pastejo foi o contínuo para manter a altura do dossel em 15 cm ± 10%. A taxa de expansão de colmos aumenta 0,000027 cm graus-dia^{-1} para cada kg de N aplicado (modelo linear). De acordo com o modelo não linear, o maior valor de taxa de expansão (0,0226 cm graus-dia^{-1}) foi observado com 220,1 kg ha^{-1} de N. A duração de vida das folhas ajustou-se ao modelo de regressão linear, com aumento de 50 graus-dia folha^{-1} comparando-se às doses de 0 e 225 kg ha^{-1} de N. Conforme o modelo não linear, o maior valor de duração de vida da folha (407,1 graus-dia) foi atingido com 208,8 kg ha^{-1} de N. Com a manutenção da altura do dossel em aproximadamente 15 cm de altura, recomenda-se utilizar 200 kg ha^{-1} de N em Tifton 85 cultivado em clima subtropical.

Palavras-chave: *Cynodon* spp., filocrono, perfilhos marcados, ureia.

Introduction

Low production rates in pasture areas are one of the causes of low profitability and competitiveness of animal production systems compared to other agricultural production systems (Moreira et al., 2015). The use of nitrogen fertilization in grass pastures is an alternative to intensify production systems by increasing the production of biomass. Forage accumulation process can be better understood through the effect of nitrogen fertilization on the morphogenesis of plants.

The amount of nitrogen (N) to be used, however, must respect the physiological limit of plant growth to reduce costs of fertilizers and the environmental contamination risk (Primavesi, Primavesi, Corrêa, Silva, & Cantarella, 2006). The availability of N to plants may vary depending on the amount of organic matter in the soil, which is higher in subtropical regions due to lower mineralization rate. The effect of using N can be measured by herbage accumulation rate and the consequent total production of dry matter (Salvador et al., 2016). The magnitude of this response, however, can be better understood when the measurements are performed on individual tillers.

Studies have been conducted to understand the responses of Tifton 85 subjected to management strategies. The use of nitrogen fertilization and irrigation in tropical climate tend to increase dry matter production of Tifton 85, allowing higher stocking rate (Pereira et al., 2011; Ribeiro & Pereira, 2011). Nevertheless, in subtropical climate, few studies have evaluated the response of the morphogenesis of Tifton 85 tillers to nitrogen fertilization strategies. Gomes et al. (2015) observed positive effect of irrigation and application of zero, 20, 40 or 60 kg ha^{-1} after each grazing cycle on the dry matter production. Thus, this study aimed to evaluate the effect of nitrogen levels on morphogenesis and structure of Tifton 85 cultivated in subtropical climate.

Material and methods

The experiment was conducted at the Department of Animal Science, Federal University of Santa Maria (UFSM), at coordinates 29º43’ S, 53º42’ W. The climate is Cfa, humid subtropical, according to the Köppen and Geiger (1928). The soil is classified as Paleudalf presenting the following values: pH-H_2O: 4.6; clay: 22%; P: 18 mg L^{-1}; K: 128 mg L^{-1}; OM: 2.6%; CEC pH 7: 12.1. Meteorological data for the months that comprised the trial period were obtained from the Weather Station of UFSM (Table 1).

Table 1. Average monthly rainfall and temperature during the study period and normal historical data. Santa Maria, state of Rio Grande do Sul.

	Month	
	December	January
Items	--------------------Observed values[1]------	
Rainfall (mm)	224.8	173.2
Average temperature (ºC)	23.7	25.2
	--------------------- Historical data[2]------	
Rainfall (mm)	133.5	145.1
Average temperature (ºC)	22.7	24.6

[1]12/01/2014 - 01/31/2015; [2]1961 – 1990.

The experiment comprised 10,000 m^2 total area divided into nine paddocks of similar size. In the experimental area (one hectare), with nine subdivisions (paddocks), Tifton 85 (*Cynodon* spp.) was stablished in January 2013. The experimental animals were Suffolk female lambs. The grazing method was continuous with variable stocking rate to maintain the sward height at 15 ± 10% cm. Three Suffolk lambs were stocked per paddock and a variable number of “put and take” animals were used to adjust the sward height. The stocking rate (SR; kg ha^{-1} body weight) was calculated by the sum of the average weight of experimental ewes, added to the average weight of each regulator ewe multiplied by the days they remained in the paddock and divided by the total evaluation days. In each paddock, we marked four sites of one m^2 each, in which nitrogen (N) was applied. The levels utilized were: zero; 75; 150 and 225 kg ha^{-1} N as urea, manually applied in a single dose on the first day of the study period.

For determination of morphogenetic and structural variables, we used the marked tiller technique (Carrere, Louault, & Soussana, 1997). On the sites where N levels were applied, ten tillers were marked and identified with colored plastic rings. We measured, in cm, the size of the expanded leaves, expanding and senescent, sward height and the stem length. The expanded leaves were measured from the ligule, while expanding leaves were measured from ligule of the last expanded leaf. Senescent leaves were measured only for the green part of the leaf blade. Evaluations of morphogenesis were held twice a week between 22/12/14 and 29/01/15.

The sward height was considered as the distance (cm) from ground surface to the average height of folding leaves. The stem length was measured from the ground to the height of the ligule of the last expanded leaf. The number of green leaves per tiller was determined by manual counting. The thermal sum was calculated using the equation: $ST = \Sigma(Tmd) - 10$ ºC, where $\Sigma(Tmd)$ is described as the sum of daily maximum and minimum temperatures of the period and ten is the basal temperature of growth for tropical grasses. The leaf appearance was calculated using the slope of the regression between the number of emerged leaves and the thermal accumulation of the period. Phyllochron was considered as the inverse value of the leaf appearance. The leaf lifespan was calculated by the product of phyllochron and the average number of green leaves per tiller. Leaf expansion and senescence (cm degree day^{-1}) were calculated by the ratio between the average elongation or senescence of the leaf between two consecutive evaluations and the thermal sum in the same period.

The experimental design was completely randomized, with four treatments (zero, 75, 150 and 225 kg ha^{-1} N) and nine repetitions per area. To compare the treatments, variables were subjected to normality test, linear and nonlinear regression (Broken line). The responses of the variables were modeled using polynomial function to second order. In regression analysis (PROC REG), the selection of the models was based on the significance of linear and quadratic coefficients using the Student’s t-test, at 5% level. Means were also subjected to nonlinear regression (PROC NLIN). Analyses were performed using the software Statistical Analysis System [SAS] (2004).

Results and discussion

The average temperature and monthly rainfall were 24.4°C and 199 mm, respectively, during the months that comprised the experimental period. Meteorological data showed that the average temperature observed was similar to the historical average temperature (23.6ºC). Rainfall values were higher than historical averages by 68% (91.3 mm; December) and 19.3% (28.1 mm; January). Temperature and rainfall were not limiting for the development of Tifton 85.

The sward height was maintained at 14.3 ± 1.1 cm and similar in all paddocks used to evaluate N levels ($p > 0.05$) and was in agreement with the experimental protocol. This sward height does not enable limitation in forage intake by sheep grazing on Tifton 85 (Carnevalli et al., 2001). To keep the height of the sward similar in all paddocks, we maintained a stocking rate of 2,039 kg ha^{-1} body weight.

The stem expansion of Tifton 85 fitted to the linear model (Figure 1) and broken line according to N levels (Table 2). In the broken line model, the highest stem expansion (0.0226 cm $degree\text{-}day^{-1}$) was found when applied 220.1 kg ha^{-1} N ($r^2 = 0.25$). The adjustment of stocking rate to keep the sward height similar in all paddocks can probably explain the similar length of stems (11.0 ± 2 cm; $p > 0.05$). Because of this effect of N on the stem expansion, the increase in stocking rate after nitrogen fertilization can represent a management strategy to prevent the accumulation of this component in the forage mass.

The stem expansion estimated by linear regression is 0.0164 and 0.0224 cm $degree^{-1}$ according to levels zero and 225 kg ha^{-1} N, respectively. The value of stem expansion estimated by linear model (225 kg ha^{-1} N) is similar to the maximum value determined by the Broken line model (0.0226 cm $degree\text{-}day^{-1}$). This effect of N on stem expansion in Tifton 85 was expected. It was observed that, even without using additional N, the stoloniferous growth of this grass was maintained, with the internodes and leaf blades comprising the aerial organs of the plant. The N available in the soil allowed a stem expansion at 0.015 cm $degree\text{-}day^{-1}$, equivalent to 0.4 cm day^{-1}, according with the average temperature in the period.

Leaf expansion and senescence, leaf appearance and phyllochron of Tifton 85 have not changed with the use of N (Table 2). The duration of leaf elongation (126.7 ± 14.6 $degree^{-1}$ days) of Tifton 85 was similar in all levels of N. The length of the expanded leaf blades (5.6 ± 1.0 cm) was not changed with the use of N ($p > 0.05$).

The similarity found in leaf appearance and phyllochron was expected because N has little effect on these characteristics, which are more dependent on temperature. Indirectly, the largest input of N could increase the leaf appearance with a reduction in phyllochron caused by the acceleration in the stem expansion. When there is high availability of N and increased stem expansion, leaf appearance is increased because a new leaf is pushed out of the sheath of the previous leaf. This effect of stem expansion on leaf appearance, however, was not observed and the amount of organic matter present in soil, equivalent to 26 kg of organic matter for each ton of soil may be a possible explanation. This amount of organic matter with mineral N available in the soil may have provided a sufficient N availability to allow stem expansion at a rate of 0.015 cm $degree\text{-}day^{-1}$, resulting in similar leaf appearance and phyllochron.

The lack of effect of N on leaf expansion may be related to the stoloniferous growth of Tifton 85, which results in increased production of stems per unit N absorbed at the expense of leaf elongation. The increase in leaf expansion is usual in species with tufted growth habit, the main effect of N observed in those species. The value of the stem expansion in the absence of nitrogen fertilization can confirm the hypothesis that the N available in the soil did not limit the leaf expansion, indicating that in the partition of absorbed N, it was intended for the expansion of stems.

Table 2. Morphogenetic and structural variables of Tifton 85 fertilized with nitrogen.

	-------Levels of N[1]-------						
Variable	0	75	150	225	L[2]	BL[2]	CV[3]
Leaf expansion[4]	0.028	0.030	0.032	0.032	ns	ns	19.7
Leaf lifespan[5]	356.5	375.2	392.7	407.1	0.0048	0.0197	10.9
Stem expansion[4]	0.015	0.019	0.019	0.022	0.0020	0.0089	24.0
Leaf appearance[6]	0.0175	0.0173	0.0176	0.0168	ns	ns	6.5
Phyllochron[5]	59.8	60.9	59.4	61.9	ns	ns	7.2
Leaf senescence[4]	0.015	0.019	0.019	0.022	ns	ns	19.9

[1]kg of N ha^{-1}; [2]Probability of N levels L=Linear models BL= Broken line; [3]Coefficient of variation (%); [4]cm $degree\text{-}day^{-1}$; [5]Degree-day; [6]Leaf $Degree\text{-}day^{-1}$; ns=Non-significant.

The maintenance of the same sward height under different levels of N may have been responsible for maintaining the same leaf senescence and duration of leaf elongation. This hypothesis is confirmed by Pereira et al. (2011), who found that leaf senescence increased by 0.30 mm day^{-1} for each cm increase of sward height. The amount of radiation incident on leaf blades at the bottom of the sward is dependent on the sward height, and increased competition for light results in increased leaf senescence.

In addition to the sward height, similar leaf senescence may explain the lack of effect of additional N on the leaf expansion. When the increased availability of N results in increased leaf expansion, there may be increased renewal of the plant leaf tissues, thus increasing the internal cycling of N, due to the greater demand for this nutrient, and consequently increasing senescence rate. Probably, the N used by the plant to increase the stem expansion has not been derived from remobilization of N from the older leaves, but rather from the root N uptake from the soil. Pereira et al. (2011) found a reduction in leaf senescence with increasing nitrogen fertilization, and attributed this effect to longer leaf lifespan and lower internal remobilization of N.

The observed similarity in the duration of leaf expansion with N fertilization can be explained by the maintenance of the same sward height in all paddocks. This height, which resulted in similar height of stems, may have provided similar length between the apical meristem and end of the stem formed by sheaths of older leaves (Difante, Nascimento Júnior, Silva, Euclides, & Montagner, 2011), requiring similar thermal accumulation for the leaf to complete its expansion. The similarity found in the leaf expansion and the duration of leaf expansion of Tifton 85 resulted in similar length of leaf blades in tillers subjected to different levels of N. This hypothesis is consistent with the data of Santos et al. (2011), who observed a longer length of stems at the highest sward heights, resulting in increase of 74% in the final length of the leaves when the sward height ranged from 10 to 40 cm.

The life lifespan fitted to linear regression (Figure 1) and broken line models according to N levels (Table 2). With broken line model, the greatest value of leaf lifespan (407.1 degree-day) was achieved when applied 208.8 kg ha^{-1} N (r^2 = 0.21). The number of expanding (2.0 ± 0.1), senescent (1.5 ± 0.2), expanded (4.5 ± 0.6) leaves and the total number (8.0 ± 0.6) of leaves were not changed with the use of N ($p > 0.05$). Probably the number of leaves of tillers was primarily influenced by the genotype of Tifton 85. This hypothesis was confirmed by Gomide and Gomide (2000), who claimed that the number of living leaves per tiller is a characteristic that tends to be constant.

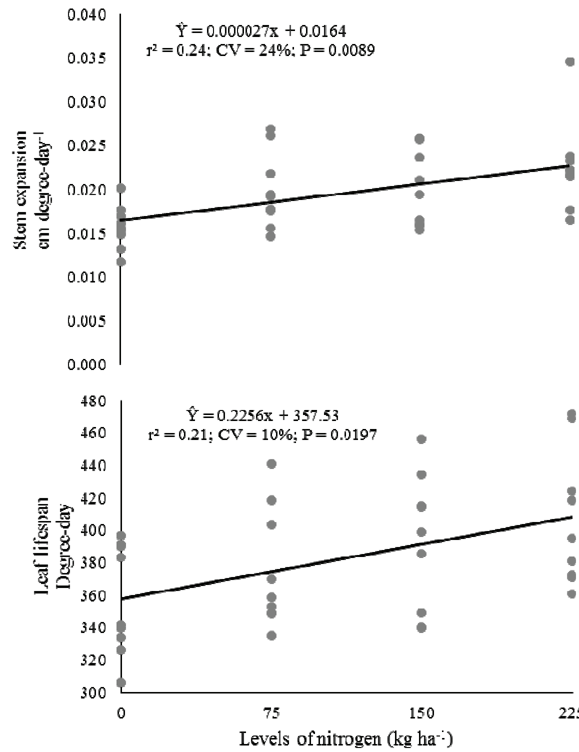

Figure 1. Stem expansion and leaf lifespan of Tifton 85 cultived in subtropical climate and fertilized with nitrogen.

The greatest value of leaf lifespan (407.1 degree^{-1} day, 16.6 days) estimated by the broken line model was similar to the value (407.8 degree day^{-1}, 16.7 days) estimated by the linear model when applied 225 kg ha^{-1} N. The magnitude of the effect of N for the duration of the leaves was not enough to alter the leaf senescence rate of Tifton 85. According to the model, only 20% of the variation in the lifespan of leaves is explained by N levels.

The use of 225 kg ha^{-1} N, compared to the level zero of N, caused an increase of 50 degree^{-1} day in the leaf lifespan, as estimated by linear regression model, equivalent to about two days, according with the average temperature of the period (16.6 vs. 14.6 days, respectively). The increase in the leaf lifespan with the use of N can increase the efficiency of harvest of leaf blade by grazing animals, as it increases the likelihood of collection of the leaf. Gastal and Lemaire (2015) defined the efficiency of utilization of the leaf as the proportion of leaf tissue produced by the plant, which is harvested by grazing animals before the onset of senescence.

The efficiency of utilization of the leaf was assessed when heifers were maintained exclusively on Alexandergrass, in which the leaves are grazed 7.14 times before the onset of the senescence process (Eloy et al., 2014). Using this value determined by these authors on the possible number of grazing cycles before the leaf blade start the senescence process, the increase of two days in leaf lifespan of Tifton 85 allows each leaf blade to be grazed more 0.3 times, allowing more efficient collection of leaf blades.

The longer leaf lifespan provided by the use of N can increase the interval between consecutive occupations in the same paddock when working with intermittent grazing, using the leaf lifespan as a management criterion, without increasing the losses by senescence. The increase in leaf lifespan when applied higher levels of N could be accompanied by a greater number of green leaves of Tifton 85 allowing the tiller to achieve the highest number of green leaves when reached the longest leaf lifespan. Nevertheless, this was not verified, because, regardless of the N level, the number of leaves of different types was kept similar in tillers.

Conclusion

The stem expansion and leaf lifespan of Tifton 85 cultivated in subtropical climate increase with 210 kg ha^{-1} of nitrogen fertilization. Phyllochron, leaf appearance, leaf expansion rate and structural characteristics of Tifton 85 are not changed by nitrogen fertilization. With sward height at approximately 15 cm, it is recommended to use 200 kg ha^{-1} N in Tifton 85 cultivated in subtropical climate.

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