Scientific Paper

Effect of planting density and regrowth age on the production and bromatological composition of *Tithonia diversifolia* (Hemsl.) A. Gray

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Abstract

Objective: To evaluate the effect of planting density and regrowth age on the biomass production and bromatological composition of *Tithonia diversifolia* (Hemsl.) A. Gray.

Materials and Methods: A trial was conducted at the Santa Lucía Experimental Farm, of the National University of Costa Rica. A split-plot, completely randomized design was used. The planting density constituted the main plot, and regrowth age, the subplot, with three repetitions per treatment. The planting densities 1, 2 and 4 plants/m^2 were evaluated and three regrowth ages (42, 56 and 70 days) were studied, for a total of nine treatments. The variables biomass production and bromatological composition were evaluated. For the data processing a variance analysis was used.

Results: The biomass production did not show significant interaction ($p < 0,05$) between planting density and regrowth age. The density of 2 plants/m^2 and regrowth age of 70 days reached the highest production (0,46 and 0,48 kg DM/m^2, respectively). The interaction of the factors planting density and regrowth age significantly affected the bromatological composition variables. The protein contents were between 8,9 and 17,5 %; the neutral detergent fiber, between 3,27 and 42,5 %, and the *in vitro* dry matter digestibility values, between 71,6 and 85,2 %.

Conclusions: Planting density and regrowth age influenced biomass production and bromatological composition of the *T. diversifolia* forage. When it was cultivated at a density of 2 plants/m^2, the highest biomass production was achieved. Meanwhile, with 4 plants/m^2 competition for sunlight was generated, which limited biomass production.

Keywords: animal feeding, biomass production, nutritional quality, forages

Introduction

Milk production systems in the tropics base their feeding on pastures, which show during the dry season low bromatological quality and reduction in biomass production (Jiménez-Castro, 2018). This situation leads to the utilization of supplements elaborated with imported raw materials, which are of high economic value, significantly increasing production costs (Rojas-Bourillon and Campos-Granados, 2015; Narváez-Uribe, 2020).

Among the strategies to increase the productive efficiency, decrease dependence on external inputs, and improve the profitability of animal husbandry systems, methods have been studied to improve the utilization of the available forage resources, as well as evaluations and validations of materials with high forage potential, utilizable in the farms, with adequate nutritional value and low production costs (Canu *et al*., 2018). These efforts are aligned with objective number 12 of sustainable development, stated by the United Nations Organization, in which it is established that consumption and sustainable production are based on the efficient use of resources and on the promotion of sustainable lifestyles (Meira-Cartea, 2015).

Silvopastoral systems constitute a resilient alternative for animal husbandry against the challenges of climate change (Morales-Velasco *et al.,* 2016). According to Cardona-Iglesias *et al*. (2017), forage banks constitute a viable nutritional strategy for the supplementation of ruminants in the tropic, because with the use of trees or shrubs, and of both, the use of external inputs is reduced, CO_2 fixation and biodiversity increase, and the general condition of soils is improved (Buitrago-Guillen *et al*., 2018).

One of the plants used in forage banks is *Tithonia diversifolia* (Hemsl.) A. Gray, which is autochthonous from Mesoamerica. This species has agronomic and bromatological characteristics of high potential for ruminant feeding. Among them, its adaptation

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to low-fertility soils, high biomass production (approximately 55 tons per year of DM), crude protein (CP) percentages between 14,8 and 28,7 %, and dry matter (DM) degradability higher than 70 %, stand out (Arronis-Díaz and Abarca-Monge, 2017; Arias-Gamboa *et al*., 2018).

The utilization of this plant in productive systems through the implementation of forage banks or as silvopastoral system has shown positive results. With its use, the nutritional composition of the animal diet is improved and production is increased, which is translated into income improvements (Rivera *et al.,* 2015; Gallego-Castro *et al*., 2017a; Rodríguez-García, 2017; Arias-Gamboa *et al.,* 2018).

According to Merlo-Maydana *et al*. (2017), harvest age is a highly important aspect, which influences the quality of the feedstuff offered to the animals. In addition, the optimum harvest moment should be determined to find balance between nutritional value and yield. In *T. diversifolia* there are studies that prove the effect of harvest age on the yield and bromatological composition (Gallego-Castro *et al*., 2017b; Guatusmal-Gelpud *et al*., 2020).

Castillo-Mestre *et al.* (2016) analyzed the effect of planting density and regrowth age on the biomass production of *T. diversifolia*. Nevertheless, in Costa Rica, there not many studies that relate these variables to the bromatological composition. The objective of this research is to evaluate the effect of planting density and regrowth age on the biomass production and bromatological composition of *T. diversifolia*.

Materials and Methods

Location and climate. The research was conducted during the rainy season (August-October) at the Santa Lucía Experimental Farm, of the National University of Costa Rica, located in the Barva de Heredia canton, at an altitude of 1 260 m.a.s.l. The average temperature is 19.2 ºC and mean annual rainfall, 2 427 mm (IMN, 2020). Its prevailing soils are of volcanic origin or andisols (Gómez and Montes-de-Oca, 1999).

Experimental design. A complete randomized split-plot design was used. Planting density constituted the main plot, and regrowth age, the subplot, with three repetitions per treatment. The plot dimensions were 8 m wide by 14 m long (112 m^2), with a buffering zone between them of 2 m. Three planting densities were evaluated: 4 plants/m^2 (planting distance of 0,5 m between rows by 0,5 m between plants), 2 plants/m^2 (0,5 x 1 m) and 1 plant/m^2 (1 x 1 m). Each one of the 9 plots was randomly distributed in the entire land, and the three regrowth ages were evaluated in each plot (42, 56 and 70 days).

Experimental treatments. Nine treatments were evaluated, regarding the combination of the factors planting density and regrowth age. In table 1 the treatments are described.

Experimental procedure. The research was carried out in a plantation of 1 288 m^2. The soil preparation was done through minimum tillage. No herbicides, fertilizers or irrigation were applied. Planting was performed through stakes (vegetative seed) 0,3 m long and 0,03 m of diameter, taken from the first and second third of the stem, with four or five buds of *T. diversifolia,* ecotype INTA Quepos, with age of 120 days.

The experimental period was 190 days: 120 for the establishment stage and 70 for data collection. The homogenization cut was done at 120 days in all the experimental plots, at a height of 0,2 m from the soil.

Biomass production. Forage samples were taken from 10 plants per subplot, at a height of 40 cm, according to the recommendations made by Arronis-Díaz (2015). The plants were randomly selected, excluding the edges of each of the experimental plots. The vegetative material (leaf and stems) of each plant was weighed with a digital scale (accuracy ± 0,001 kg). These data were used to estimate the biomass production in DM, expressed in kg DM/m^2. The determinations were carried out in the Laboratory of Animal and Plant Product analyses (LAPAV), from the School of Agricultural Sciences of the National University of Costa Rica.

Bromatological composition. The plant material obtained from the 10 *T. diversifolia* plants harvested in each subplot, was chopped and mixed to collect a sample of 1 kg of fresh forages (leaves and stems). The samples were packaged in plastic bags, duly identified, and were transferred to the LAPAV to determine the variables DM, CP, ethereal extract (EE) and ashes (Ash) according to the AOAC (1998). For determining the acid detergent fiber (ADF) and neutral detergent fiber (NDF), the methodology described by Van Soest and Robertson (1985) was used. The *in vitro* dry matter digestibility (IVDMD) was calculated according to the indications by Van Soest *et al*. (1966).

Statistical analysis. Variance analysis was carried out for split-plots, according to the description made by Kaps and Lamberson (2004). The means were contrasted through Tukey's test at 5 % of significance. The fulfillment of the normality assumptions was tested by Shapiro-Wilk test, and for variance homogeneity Levene's test was applied. The data were analyzed with the statistical program SAS®, version 9.0 (SAS Institute Inc., 2009).

Results and Discussion

Table 2 shows the biomass production values of the *T. diversifolia* forage. No significant interaction effect ($p < 0,05$) was observed between planting density and regrowth age. However, significant differences ($p < 0,05$) were obtained between densities and regrowth ages, where increase was observed in production, depending on the increase of age and planting density.

The highest biomass production values were found in the densities of 2 and 4 plants/m^2, without showing significant differences between them. The highest density was reached when sowing 2 plants/m^2, with value of 0,46 kg DM/m^2, which would be equivalent to 4,60 t DM/ha per cut. Significant differences were found for the planting density of 1 plant/m^2 with regards to the other two evaluated densities.

The results of this study are similar to the ones obtained by Ríos and Salazar (1995), who found effect of planting density on the forage production of *T. diversifolia* plants. These authors reached the best yields in the densities higher than 1 plant/m^2. The planting density with higher biomass production was that of 1,80 plants/m^2, which coincides

Table 1. Experimental treatments with regards to the effect of planting density and regrowth age of *T. diversifolia*.

Treatment	Planting density, plants/m^2	Days of regrowth
1	1	42
2	1	56
3	1	70
4	2	42
5	2	56
6	2	70
7	4	42
8	4	56
9	4	70

Table 2. Biomass production of *T. diversifolia,* according to planting density and regrowth age.

Variable	Treatment	DM production, kg/m^2
Plant density/m^2	1	0,24^b
	2	0,46^a
	4	0,42^a
P - value		<0,009
SE ±		0,035
Regrowth age (days)	42	0,25^c
	56	0,39^b
	70	0,48^a
P - value		<0,0001
SE ±		0,028
Density x Age	P - value	0,3732

a, b y c: Different letters in the same column significantly differ for $p < 0,05$ (Tukey).
SE: standard error

with the results of this research. Ríos and Salazar (1995) add that it would be possible to obtain higher productive yields in densities higher than 2 plants/m^2, although this could mean possible plant health risks, due to the high planting density.

In this research there was increase of biomass production, as the regrowth age increased. The mean DM production values for each regrowth age showed significant differences ($p < 0,05$), being 0,25; 0,39 and 0,48 kg DM/m^2, for 42, 56 and 70 days, respectively (table 2). The highest biomass production in DM was reached at 70 days of regrowth, with 0,48 kg DM/m^2.

These results are in correspondence with Arias (2018), who reported 0,42 kg DM/m^2 at 50 days of regrowth, under equal edaphoclimatic conditions. The DM production increased significantly ($p < 0,05$) as regrowth age increased. This situation is also very similarly referred in the results reported by Guatusmal-Gelpud *et al*. (2020).

In different works, in which the biomass production of *T. diversifolia* in DM can vary from 0,10 kg DM/m^2 to 0,42 kg DM/m^2 depending on the edaphic and climate conditions (Castillo-Mestre *et al*., 2016; Arias-Gamboa, 2018; Guatusmal-Gelpud *et al*., 2020), comparable results to the ones in this study are reported.

The significant increase ($p < 0,05$) in production per square meter with regards to the evaluated regrowth ages, is ascribed to the fact that plants have more time to develop their tissues (vascular, meristematic, and foliar). For such reason, the increase of fresh matter biomass has direct repercussion on the dry biomass production (Elizondo-Salazar, 2017).

Bromatological composition. The variables of bromatological composition showed significant interaction ($p < 0,05$) between planting density and regrowth age. It is important to mention that, in this study, in none of the evaluated regrowth ages, the plants reached flowering or signs of being approaching it.

The DM contents of the evaluated treatments are shown in table 3. The values oscillated from 13,4 to 16,9 %, results that are in agreement with the report by Rodríguez-García (2017). This author refers that the DM percentage of *T. diversifolia* varies from 13,5 to 35,0 %, according to the agroecological conditions and crop management.

The highest DM content was obtained for the density of 2 plants/m^2, with 70 days of regrowth (16,9 %). These values are close to the ones reported by Arias-Gamboa (2018), who achieved 15 % of DM in *T. diversifolia*, at planting density of 1,2 plants m^2 and 50 days of regrowth. The DM content was very similar to that informed by Argüello *et al*. (2020), when evaluating the forage from this material in the dry and rainy season (19 %).

Table 3 shows that as regrowth age increases, the DM content increases. This performance has been described in works that evaluate this variable in *T. diversifolia*, at different regrowth ages (Guatusmal-Gelpud *et al*., 2020). It is normal performance in most forages, in which the DM contents increase,

Table 3. Bromatological composition of *T. diversifolia*, according to the different planting densities and regrowth ages.

Density, plants/m2	Regrowth age, days	DM	CP	NDF	ADF	IVDMD	Ash	Ethereal extract
1	42	13,4^c	17,5^a	33,1^d	23,9^{cd}	85,2^a	16,3^a	5,4^a
	56	16,0^a	12,5^c	39,2^b	29,0^b	81,9^b	13,0^b	3,3^b
	70	16,3^a	9,0^e	41,2^a	31,0^a	74,1^d	12,8^b	2,1^c
2	42	13,5^c	15,9^b	32,7^d	22,6^d	82,9^{ab}	15,3^a	5,1^a
	56	16,9^a	10,8^d	34,0^d	23,8^{cd}	79,9^{bc}	12,9^b	3,1^b
	70	16,9^a	8,9^e	35,7^c	25,2^c	71,6^d	11,5^c	2,0^c
4	42	13,9^{bc}	15,3^b	42,5^a	31,7^a	84,7^{ab}	15,5^a	5,4^a
	56	15,9^{ab}	10,78^d	38,1^b	29,3^b	82,3^b	13,2^b	2,9^b
	70	16,2^a	9,6^e	39,1^b	29,2^b	77,6^c	12,8^b	2,1^c
SE ±	0,980	0,490	0,706	0,600	13,975	0,499	0,279	
P - value	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,012	

CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, IVDMD: *in vitro* dry matter digestibility
a, b, c: Different letters in the same column are significantly different $p < 0,05$ (Tukey)
SE: standard error

as the regrowth age increases (Elizondo-Salazar, 2017).

In that sense, the effect caused by the regrowth age was different, according to planting density. When planting was done at 1 and 2 plants/m^2, there was lower DM content at 42 days in the treatments of 56 and 70 days, which did not differ significantly between them.

In the density of 4 plants/m^2, there were no significant differences ($p < 0,05$) in the DM content between the regrowth ages of 42 and 56 days. This result could be related to higher competition for the search for light among the plants. Castillo-Mestre *et al*. (2016) also reported this physiological response, when studying different planting densities (1; 1,3 and 2 plants/m^2) in *T. diversifolia*. These authors described that, at higher planting density, the stems grew vertically, only with foliage in the canopy, which caused higher fiber content in the plants, with subsequent effect on the DM content.

Table 3 shows the results of CP, which were between 8,9 and 17,5 % for the plant *T. diversifolia* at the different evaluated planting densities and regrowth ages. The highest value of this variable was reached in the treatment of 1 plant/m^2 and 42 days of regrowth (17,5 %). *T. diversifolia* showed the lowest CP percentages in the three planting densities, at the age of 70 days. In this case, values of 9,0; 8,9 and 9,6 % were reached for the densities of 1, 2 and 4 plants/m^2, respectively, without significant differences among them. Meanwhile, the highest values were found in the regrowth of 42 days, with values of 17,50; 15,9 and 15,3 % for the densities of 1, 2 and 4 plants/m^2, the CP being significantly ($p < 0,05$) higher in the density of 1 plant/m^2 (table 3).

Arronis-Díaz and Abarca-Monge (2017) reported in *T. diversifolia* CP contents of 14,8; 9,4 and 10,6 %, with regrowth ages of 40, 50 and 60 days, respectively, values that are very close to the ones found in this study.

Table 3 shows that the CP content decreases as the regrowth age increases, with percentages of 17,5, 12,5 and 9,0 at the ages of 42, 56 and 70 days, respectively, in the planting density of 1 plant/m^2. A similar performance appeared in the planting densities of 2 and 4 plants/m^2, but with lower CP contents at the ages of 42 and 56 days. This effect could be related to the modification of the growth pattern in the plants, of 2 and 4 plants/m^2, with regards to the density of 1 plant/m^2. In this arrangement of density, the plant could have shown higher proportion of leaf, which is the part that has higher CP contents with regards to the stems (Elizondo-Salazar, 2017). In addition, it could show less competition for sunlight, which influences the stem elongation process.

The NDF contents found in this research were between 32,7 and 42,5 % (table 3). In the planting density of 1 plant/m^2, there was significant increase ($p < 0,05$) in the NDF values (33,1; 39,2 and 41,2 %) at the ages of 42, 56 and 70 days of regrowth, respectively. In the density of 2 plants/m^2, values of 32,7 and 34,0 % were recorded for the ages of 42 and 56 days, and there was a significant increase, which reached 35,7 % for the regrowth of 70 days. Regarding the density of 4 plants/m^2, the highest NDF content was 42,5 %, at the age of 42 days, which was significantly higher than the one found in the regrowth of 56 and 70 days.

Similar data were reported by Cardona-Iglesias *et al.* (2017), who obtained 38,4 and 39,0 % of NDF for plants with 90 and 70 days of regrowth, respectively. In turn, Verdecia *et al*. (2011) reported NDF contents of 43,7 and 46,8 %, for regrowth ages of 60 and 120 days, respectively. These results, slightly higher than the ones found in this study, could be associated to agronomic management factors. At the age of 120 days, there was higher deposit of cell wall components.

The NDF content increased, as the regrowth ages increased in the densities of 1 and 2 plants/m^2. Nevertheless, its performance in the density of 4 plants/m^2 was different, the highest fiber percentage being obtained at the age of 42 days (42,5 %). This effect could be related to the modification of the growth pattern of plants, in an arrangement of higher planting density and early vegetative states (42 days), because under these conditions the elongation of stems could be higher, due to competition for sunlight.

This performance was no observed after 42 days of regrowth, because the growth pattern could have been modified and shown higher leaf proportion, and lower proportion of stems. This would explain lower NDF content, at the ages of 56 and 70 days, with regards to the age of 42 days of regrowth.

The ADF contents reached values between 22,6 and 31,7 % (table 3), with a similar performance to that of NDF in the densities of 1 and 2 plants/m^2, the ADF value was higher as the regrowth age increased, being significantly ($p < 0,05$) different. This performance is similar to the one described by García-Marrero (2003), who indicates that the plant age influences the fibrous components of forages.

In the planting density of 4 plants/m^2, a different performance was observed. There was higher ADF percentage, at the age of 42 days (31,7 %). Just like in the NDF, this performance could have been related to the modification of the growth pattern in the plants with the highest density. In them, competition for sunlight could cause higher stem proportion, and thus, the accumulation of fibrous material.

In this study, the ADF values are in agreement with the report by Cardona-Iglesias *et al.* (2017), who referred in *T. diversifolia* 27,2 % of ADF, at 70 days of regrowth. Nevertheless, Arronis-Díaz and Abarca-Monge (2017) recorded ADF contents of 29,40; 42,90 and 37,00 % for 40, 50 and 60 days of regrowth, respectively, values that are higher than the ones in this research.

The IVDMD values showed trend to decrease, as the regrowth age increased in all the evaluated treatments, which does not differ from the performance described by Soto *et al.* (2009). This could be related to the fiber contents found in this work, where the values oscillated, generally between 71,6 and 85,2 % in the evaluated treatments (table 3).

In the densities 1 and 2 plants/m^2, as the fiber content decreased, the IVDMD increased. This did not occur in the arrangement of 4 plants/m^2, where at 42 days there was higher fiber percentage, performance that could be related to higher fiber digestibility.

In the density of 1 plant/m^2 significant differences ($p < 0,05$) were observed among the three evaluated regrowth ages (table 3). This performance was different when 2 and 4 plants/m^2 were cultivated, where there was only significant difference ($p < 0,05$) at the regrowth age of 70 days, compared with the other ages. This could be associated to the physiological factor, which modifies the general structure of the plant, due to the effect caused by the competition for sunlight. In the planting density of 1 plant/m^2, there was a normal performance. When the regrowth age increased, the IVDMD significantly decreased ($p < 0,05$).

In the densities of 2 and 4 plants/m^2, the effect of the competition for sunlight could have generated a modification in the normal performance of IVDMD of the plant (lower leaf/stem ratio), for which significant differences did not appear between the ages of 42 and 56 days. After 56 days, significant influence ($p < 0,05$) of regrowth age was observed in the densities of 2 and 4 plants/m^2.

This performance was described by Noda and Martín (2008), who used three planting densities (1,25; 2,50 and 3,80 plants/m^2), and found that at higher densities morphological changes occur in the plants, due to the competition for sunlight.

The IVDMD values are similar to the ones obtained by Arias-Gamboa (2018), who refers 73,5 % of IVDMD, when evaluating the whole plant of *T. diversifolia*, at a regrowth age of 50 days, and planting density of 1,20 plants/m^2, under the same agroclimate conditions of this study. These values were also similar to those reported by Ruíz *et al.* (2016). These authors found values from 72,3 to 79,8 % of *in vitro* apparent DM digestibility, and from 81,1 to 85,7 % of true digestibility. The ash values were between 11,5 and 16,3 % in the different evaluated treatments (table 3).

In all the density arrangements, there was higher ash content at 42 days, with regards to the other regrowth ages. According to Jaramillo-Jaramillo and Seberino-Mondragón (2015), the ash content of forages decreases as the regrowth age increases, because the DM content of plants increases faster than mineral absorption, which causes many minerals to decrease their concentration.

In the density of 2 plants/m^2, values of 15,3; 12,9 and 11,5 % for the ages of 42, 56 and 70 days, respectively. These values were significantly different; while in the densities of 1 and 4 plants/m^2, the ash content did not decrease significantly ($p < 0,05$) between 56 and 70 days of regrowth age.

In this research, the ash values were very close to the ones obtained by Arias-Gamboa (2018), who indicated 11,5 %, at planting density of 1,20 plants/m^2 and 50 days of regrowth, when he evaluated the whole plant of *T. diversifolia* (stems and leaves).

The EE values oscillated between 2,0 and 5,4 %, with significant differences ($p < 0,05$) among the treatments (table 3). In each arrangement of plant density, there was reduction of the EE percentage, as the regrowth age increased. Nevertheless, higher decrease of this variable was recorded when passing from 42 to 56 days, with regards to the change observed between 56 and 70 days in the three evaluated densities. This decrease of EE agrees with the description made by Elizondo-Salazar (2017), who indicates that the content of this nutrient could vary between 3 and 10 %, and decreases with the forage age.

In this work, the EE values are similar to those reported by Arias-Gamboa (2018), who found contents of 2,40 % in *T. diversifolia*. In a study conducted in Colombia, Ruíz *et al.* (2016) found values between 0,90 and 2,05 for different *T. diversifolia* ecotypes.

Conclusions

The planting density and regrowth age influenced biomass production and bromatological composition of the *T. diversifolia* forage. When it was cultivated at a density of 2 plants/m^2, the highest biomass production (0,46 kg DM/m^2) was achieved. Meanwhile, with 4 plants/m^2 competition for sunlight was generated, which limited biomass production.

The results prove that *T. diversifolia* can be cultivated at a density of 2 plants/m^2 and can be harvested at intermediate ages, in order to maintain an adequate content of biomass production and bromatological quality.

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Conflicts of interests

The authors declare that there are no conflicts of interests among them.

Authors' contribution

- Luis Diego Paniagua-Hernández. Design and setting up of the experiments, data collection and analysis in the field.
- Luis Mauricio Arias-Gamboa. Design and setting up of the experiment in the field, data collection and processing, research advisory, manuscript writing and revision.
- Andrés Alpízar-Naranjo. Design and setting up of the experiment in the field, data collection and processing, research advisory, manuscript writing and revision.
- Miguel Ángel Castillo-Umaña. Design and setting up of the experiment in the field, data collection and processing, research advisory, manuscript writing and revision.
- María Isabel Camacho-Cascante. Collaboration in the design and setting up of the experiments, research advisory, manuscript writing and revision.
- José Enrique Padilla-Fallas. Design and setting up of the experiments, data collection and analysis in the field.
- Manuel Campos-Aguilar. Design and setting up of the experiments, research advisory, manuscript writing and revision.

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