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Abstract

The objectives of this study were to evaluate the growth curve and chemical composition of *Cratylia argentea*. The experiment was conducted with *Cratylia* planted in two-line spacings of 2 or 3 meters between rows and 0,5 m between plants. *Cratylia* plants were evaluated in a cycle of 126 days, with pruning events every 21 days, and a total of 6 assessments for each treatment. Parameters of bromatological composition (DM, CP, NDF, ADF) of plant edible fraction (EF) and productivity (total productivity and productivity of the EF in DM) were evaluated. The study was performed in a completely randomized design with 2 treatments (spacing) and 6 replications. The variations over days were evaluated using regression analysis, and the results were compared by Fisher test ($p < 0,05$). The models that had the best fit for productivity was exponential and logistic, whereas, for the bromatological composition it was Gompertz and polynomial. The 3 m spacing had lower productivity and CP content. The *Cratylia* growth rate reduced with increased plant maturity, which suggested its prune at 86 days for the 2 m spacing and 91 days for 3 m spacing. Based on the results found in this research, the 2 m showed greater yield and CP content. Both spacings presented values of CP, NDF, and ADF of 20.26, 60.29 and 40.63% for 2 m spacing and 18.97, 60.61 and 42.11% for 3 m spacing, respectively for 105 days of pruning, which was less productive for both spacings.

Keywords

Cratylia argentea, tropical legumes, forage shrubs, silvopastoral systems, agroecology,

Statements and Declarations

The authors declare they have no conflict of interest.

Author Contribution

All authors contributed to the study conception and design. Conceptualization, Investigation, Material preparation, Visualization was performed by Leonardo Campos Teatini Climaco, Angela Maria Quintao Lana and Elaine Cristina Teixeira. Data collection were performed by Leonardo Campos Teatini Climaco, Lucas Freires Abreu, Walter Jose Rodrigues Matrangolo and Elaine Cristina Teixeira. Data analyses were performed by Angela Maria Quintao Lana and Fernando Antonio Souza. The first draft of the manuscript was written by Leonardo Campos Teatini Climaco and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Introduction

Cratylia (*Cratylia argentea*) is a perennial shrub with high foraging potential. It is easily spread through seeds and is a leguminous plant, which performs biological nitrogen fixation (BNF) and contains high levels of crude protein (CP) (Matrangolo et al., 2018; Panadero et al., 2020). Due to its nutritional value, the species is promising for use in feeding different animal species, mainly ruminants (Mora et al.,

2017; Silva et al., 2018). Sarria and Martens (2013) also found promising results evaluating the quality of *C. argentea* silage for swine feeding. However, studies that seek to maximize its use in livestock are scarce, and the proper management of forages requires deep knowledge about the physiology of the plant and its growth habit (Machado Filho et al., 2021).

The increased forage use efficiency requires an understanding of its optimal herbage accumulation to obtain greater biomass productivity. This optimal herbage accumulation (also known as optimal resting period) should ensure the plant restores its nutrient reserves to ensure successive regrowth, without decreasing productivity over the growing season (Machado Filho et al., 2021). A proper management at the optimum resting period should favor the plant perenniality, since it contributes to maintain its carbohydrate reserves (Machado 2010; Machado Filho et al., 2021). Furthermore, the greater efficiency of biomass production, especially when this production is not dependent on high doses of chemical fertilization, represents an increase in the fixation of greenhouse gases (GHG) (Machado Filho et al., 2021). This happens due to an increased photosynthetic efficiency, leading to greater fixation of carbon dioxide (CO₂) in plant biomass (Cain et al., 2019; Viglizzo et al., 2019).

It is essential to understand the nutritive value of potential forage plants to determine the best practices, according to animal category. In this case, the bromatological composition is the parameter used to evaluate the nutritional value of forages (Canul-Solis et al., 2020), since they affect the consumption, digestibility, and performance of the animals (Pereira et al., 2018). Thus, given the potential of *Cratylia* for animal feed, it is essential to study the adaptation of the species in an intensive cultivation system.

The study of growth curves and their models is a widespread approach to analyze the productive efficiency of crop biomass (Silva, 2010) from a biological perspective. These models should have parameters whose biological interpretation help understanding the phenomena that occur during growth (Cao et al., 2019). In addition, regression models for the growth curve allow the estimation of accumulated biomass value for different plant ages within the evaluation interval (Cao et al., 2019; Silva, 2010).

The biomass accumulation is determined by several factors, specific processes, and relationships (Cao et al., 2019). In general, it is composed of an initial phase of slow growth, which is followed by an exponential growth phase that ends with a stabilization phase, which growth continues, but with a decreased growth rate (Di Crescenzo et al., 2021).

The current research aimed to better understand the ecophysiology of *Cratylia*, considering some criteria such as: (1) determining the optimal herbage accumulation (2) evaluating the effects of plant spacing on productive parameters and nutritional quality and (3) follow the evolution of the chemical composition over the days. The objective was also to maximize the productive potential of the *Cratylia*, based on the understanding of growth curve and its use in the optimal herbage accumulation timing.

Materials and Methods

The experiment was conducted at the experimental station of Embrapa Milho e Sorgo in Sete Lagoas, MG, Brazil, located at 19°28' 40°15' of Greenwich and an altitude of 732 m above sea level (Campos et al., 2019).

The soil is classified as typical dystrophic Red Latosol with clayey texture with deep, porous, and great aggregate stability. Its structure is of the small and very small granular type, with a solid appearance

and very well drained. The predominant vegetation is the *Cerrado* (Albuquerque Filho et al., 2022; Santos et al., 2018).

The soil characterization in the experimental area was divided into a strata of 0-20 cm, 20-40 cm, 40-60 cm. Organic matter levels, which are the basis for nitrogen fertilization, were considered good for all evaluated ranges with values of 3.44, 2.89, and 2.87 dag/dm³, for 0-20, 20-40 and 40-60 cm strata, respectively. Phosphorus (P) levels were considered very low for all ranges with 2.18, 1.43 and 1.62 mg/dm³ for 0-20, 20-40 and 40-60 cm strata, respectively. The potassium (K) levels were considered low with 39.26, 19.98, and 17.20 mg/dm³ for 0-20, 20-40 and 40-60 cm strata, respectively. Base saturation (V %) was considered low for the 0-20 and 20-40 cm ranges with values of 30.51 and 25.65%, and very low for 40-60 cm with values of 15.71%. The exchangeable acidity (Al₃⁺) was very low for the 0-20 and 20-40 cm ranges with values of 0.14 and 0.20 cmolc/dm³, respectively and medium for the 40-60 cm range with values of 0.52 cmolc/dm³ (Ribeiro et al., 1999).

The experimental area had been managed with conservationist practices, without soil disturbance and with input of pruned biomass, which contributes to the maintenance of soil nutrients (Oliveira et al., 2021). Mattar et al. (2020) evaluated *Cratylia* accessions in the Terra Ronca State Park, Goiás. *Cratylia* individuals were found in different soils, such as cambisols, argisols and lithosols. The P levels found in the soil are considered low over the entire sampled area.

The climate in the region is Cwa type (according to the Köppen classification) considered as mesothermal with dry winter and hot summer. It is characterized by an average temperature below 18°C in the coldest month, and in the hottest month above 22°C. The total precipitation of the driest month was less than 30 mm. The average (annual) air temperature in the region is 22°C and the average annual precipitation is 1340 mm (Campos et al., 2019).

The meteorological station located at the Embrapa Milho e Sorgo in Sete Lagoas, MG, provided data on rainfall and average air temperature throughout the experimental period. During the *Cratylia* growth cycle the area received a rainfall of 1,260 mm and the average temperature was approximately 23°C. The period before the harvest was composed of a sequence of low temperatures and little rainfall. The distribution of temperature and precipitation in the experimental area is illustrated on Figure 1.

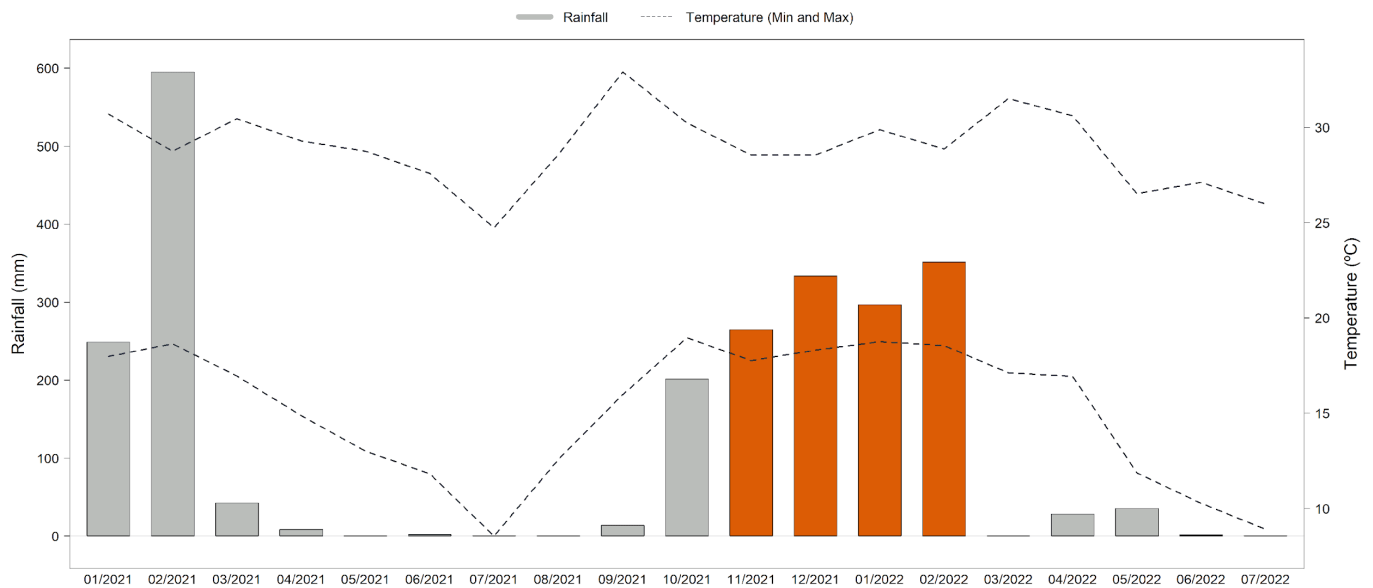


Fig. 1 Temperature and rainfall from January 2021 to July 2022. Orange bars represent the rainfall during the experimental period.

The experiment was conducted over a period of 126 days (from November 2021 to March 2022). *Cratylia* seedlings were transplanted in 2009 with spacing of 0.5 m between plants and spacing of 1, 2, 3, 4, and 5 m between rows, in an area of 450 m² (30 x 15m). For this experiment, only plants with spacing of 2 and 3 meters between the rows were used. The area was managed with periodic pruning of the plants, and the last standardization pruning carried out on July 20th, 2021.

A harvest was made at 50 cm from the ground to standardize the *Cratylia* plants at the beginning of the experiment and at the end of each experimental cycle. The sampling unit consisted of 6 plants in each harvest, for each spacing used (using spacings of 2 x 0.5 and 3 x 0.5 m), totaling 72 evaluated plants. In each treatment, the plants were evaluated within the rows at the time of standardization. The plants that were closest to the average condition of the population were selected, eliminating outliers, edges, and those with flaws in the adjacent lines. After selecting the plants that were part of the experiment, a random draw was made to assign the date which a sample unit would be evaluated.

Sections of *Cratylia* harvest individuals were made at 21, 42, 63, 84, 105 and 126 days of regrowth at 50 cm height. At the time of each harvest, the number of stems from each plant was evaluated, since the number of branches interferes with productivity (Matrangolo et al., 2018). The samples were weighed to evaluate the biomass production, aiming to determine the growth curve and chemical composition of each harvest. After the end of the experimental cycle, a backpack cutter model STIHL FS 160 (Andreas Stihl AG & Co, Waiblingen, Germany) was used for a standardized harvest at 50 cm from the ground.

The collected samples were fractionated into Edible Fraction (EF), which is formed by stems smaller than 5 mm and leaves, and Woody Fraction (WF), which is formed by stems and branches larger than 5 mm (Panadero et al., 2020). The samples were wrapped in brown paper and dried in a forced ventilation oven at 55°C for 72 hours. Subsequently, they were grounded at 1mm in a Willey-type mill, identified and stored in plastic pots.

The samples were used to evaluate crude protein (CP), dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF). The bromatological composition was made by the bench method in the laboratory (Silva and Queiroz, 2002).

Several regression models (polynomial, exponential, logistic, Gompertz and Richards were tested to determine the growth curve in DM, EF, NDF, ADF and CP contents as a function of the experimental days. These models are widely used to represent the plant growth curve (Cao et al., 2019; Di Crescenzo et al., 2021). The models were adjusted for each level of the evaluated plant spacing based on a generalized linear model (GLM) and generalized nonlinear models (GNM) (Pinheiro and Bates, 2000).

The models that presented adequate adjustment to the data and significance ($P < 0.05$) for all parameters were compared according to the *Likelihood-ratio* test. Models that showed no statistical difference were subjected to the Akaike criteria (Akaike, 1974) and its best performance in the adjustment for each treatment.

Model identity analysis was performed to assess whether the estimated equations for each treatment could be replaced by a single equation, or the individual parameters differed between treatments (Pinheiro and Bates, 2000).

To compare the linear and polynomial models, the value of the coefficient of determination (R^2) regression models were also used to evaluate the growth of the edible fraction (EF). The growth of EF represents the biomass potentially usable as forage since it is the most nutritious fraction. Comparison between EF and total biomass production during the experimental period can provide information for choosing the best growth stage of *Cratylia* for animal feed. The comparison between the plant spacings for the variables %NDF, ADF, DM, CP, biomass production and EF of biomass production was performed using analysis of variance. Discrimination of means was performed using the Tukey test at $p < 0.05$.

The analyzed dataset was subjected to the Shapiro-Wilk normality test and Bartlett's homoscedasticity test ($p < 0.05$). When normality assumptions were not met, data were transformed using the Box-Cox function. The weighted least squares method was used for analysis of variance for that showed no homoscedasticity. In addition, the logarithmic transformation of the data was performed using the natural logarithm (ln), if needed.

All analyzes were performed using the R statistical analysis software (R Core Team, 2022).

Results

Growth curve

The logistic regression model for dry matter production of the entire plant (g) in both planting spacings showed the best fit, considering the parameters of the AIC criterion. As far as the EF productivity, the exponential regression in spacings 2 and 3 meters showed the best fit (Table 1).

Due to a high data dispersion, the data related to productivity (whole plant DM production and EF productivity) were transformed into logarithms in the Neperian logarithm to improve data accuracy and evaluation.

Table 1 Estimates of regression equations for the variables Prod(g)¹, Prod EF(g)², as a function of evaluation time at 2 and 3 m spacing between rows

Variable	Models	
	2 m spacing	3 m spacing
Prod ¹ (g)	$6,177 / (1 + \exp^{\frac{3}{0,064x}(2,046 - 0,064x)})$ (AIC ⁴ = 63,36)	$6,422 / (1 + \exp^{(1,619 - 0,055x)})$ (AIC = 60,73)
Prod EF ² (g)	$6,6948(1 - \exp^{(-0,0217x)})$ (AIC = 58,64)	$6,525(1 - \exp^{(-0,025x)})$ (AIC = 59,86)

¹ Prod - Total productivity per plant in g of DM
² Prod EF - Productivity of the edible fraction in g DM
³ exp – Exponential function with Base e
⁴ AIC - Akaike Information Criterion

The Prod (g) growth curve for spacings 2 and 3 m showed similar behavior. The logistic function was characterized by a low growth phase, followed by an acceleration phase and later stabilization. The 3-meter spacing had a greater initial growth and a less intense deceleration (Figure 2). Both spacings showed similar growth values in the final evaluation periods.

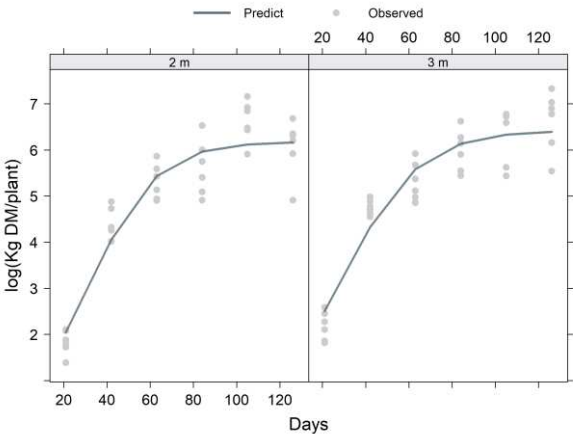


Fig. 2 Regression estimates for dry matter production per whole plant in natural logarithm (Ln) for 2 and 3 m spacing between rows over the evaluated period

As far as the regression equation for DM production of EF, both spacings showed a similar behavior, which the growth rates had high levels at the beginning of the period followed by a decrease (Figure 3). The regression equation that best fitted this variable were exponential.

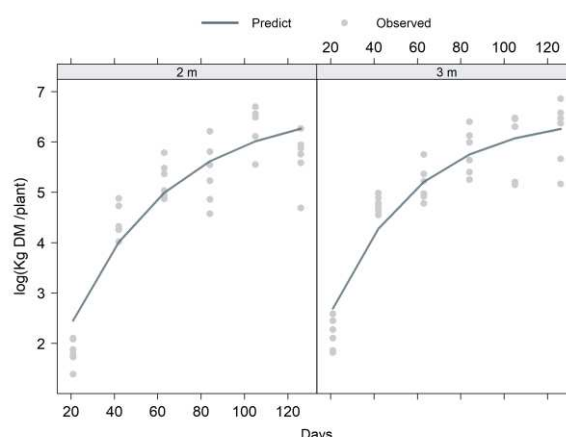


Fig. 3 Regression estimates for dry matter production of the Edible Fraction (FC) in natural logarithm (Ln) for 2 and 3m spacing between rows over the evaluated period.

In the absence of significant interaction between the factors (Spacing x Day) for Prod DM and Prod EF, the comparison was made on the average effect of the isolated factors (Spacing and Days of evaluation) (Table 2).

Table 2 Analysis of variance, estimates of means and standard error of Prod MS¹(g) Prod of EF² (g) in the spacing of 2 and 3 m between lines, regardless of the evaluation days

Variables	Spacing		SE ³	P-Value ⁴		
	2	3		Spacing	Days	ExD ⁵
Prod	92,81 b	120,66	7,3	9,25*10 ⁻³	4,51*10 ⁻	0,09
DM ¹ (g)		a	7		41	
Prod EF ²	82,79 b	106,73	6,4	0,01	1,61*10 ⁻	0,09
(g)		a	8		39	

Means followed by different letters are statistically different according to Fisher's test (P<0.05).

¹ Production mean per plant (g of DM)

² Production mean of the edible fraction per plant (g of DM)

³ Standard error

⁴ Probability value

⁵ Interaction between Spacing and Days

The 3 m spacing showed a greater production for total and EF than the 2 m spacing, regardless of the evaluation days. This absence of interaction for the productive parameters indicates that there is no differentiated response to the spacing factor over the days.

Bromatological composition

The Gompertz model showed the best fit for DM content on both spacings (Table 3). While for the other bromatological parameters (CP, NDF, ADF), polynomial models performed best. The adjustment criteria of the coefficient of determination R^2 and the parameters of the AIC criterion were considered.

Table 3 Equations of regression estimates for DM, CP, NDF and ADF contents as a function of evaluation time at 2 and 3 m spacing between rows

Variable	Regression Models	
	2 m Spacing	3 m Spacing
DM (%)	$37,771 \exp^{-1}(-\exp(1-0,0185(x+65,2167)))$ (AIC ² = 159,07)	$37,3923 \exp(-\exp(1-0,0258(x+33,4425)))$ (AIC = 161,46)
CP (%)	$0,2608-0,00062x$ (R ^{2 3} = 0,6951)	$0,292-0,001866x+0,00000824x^2$ (R ² = 0,81)
NDF (%)	$0,4554+0,002466x-0,00001x^2$ (R ² = 0,84)	$0,4425+0,003081x-0,00001435x^2$ (R ² = 0,78)
ADF (%)	$0,3312+0,0018x-0,000009468x^2$ (R ² = 0,54)	$0,3354+0,001658x-0,000008103x^2$ (R ² = 0,48)

¹ exp – Exponential function with Base e

² AIC - Akaike Information Criterion

³ R² – Coefficient of determination

At 3 m spacing, a steady drop was observed, followed by a stabilization and a slight increase in CP contents (Figure 4). It is worth noting that, the regrowth time was determinant for the CP contents for the two plant spacings. The minimum value was not reached for the 2 m spacing, and the lowest value found was at the end of the evaluation period on day 126. On the other hand, the minimum point was estimated on day 113 for the 3 m spacing.

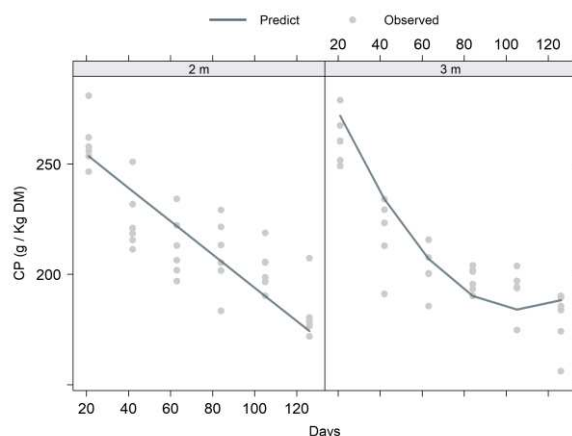


Fig. 4 Regression estimates of crude protein contents in percentage for 2 and 3 m spacing between rows over the evaluated period

Based on the estimation of polynomial regressions, the points of maximum NDF values were estimated for days 123.30 and 107.35 for spacing of 2 and 3m between rows, respectively (Figure 5). Both planting spacings showed a quadratic function with an upward concavity, which there is a growth followed by a maximum point and a fall in NDF values.

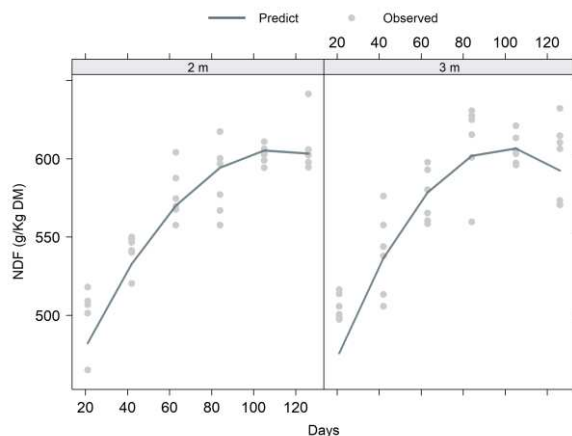


Fig. 5 Regression estimates of neutral detergent fiber levels in percentage for 2 and 3 m spacings between rows over the evaluated period

The polynomial regression estimations for ADF showed a quadratic function, which was a similar behavior to NDF for both 2 and 3 m spacings (Figure 6). However, the points of maximum ADF values were estimated at 95.06 and 102.31 for spacing of 2 and 3m between rows, respectively, which happened before the NDF.

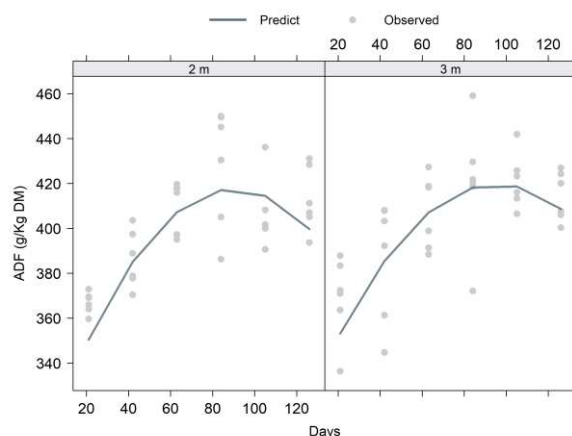


Fig. 6 Regression estimates of Acid Detergent Fiber in percentage for 2 and 3 m spacing between rows over the evaluated period

Overall, an increase in the fibrous fractions content was observed throughout the evaluations, with a stabilization tendency in the final evaluation periods. The results found for NDF and ADF were consistent with forage development. However, the decrease in the final periods after the low period was an unusual result.

Since there was no significant interaction between the factors (Spacing x Day) for the evaluated variables, the comparison was made on the average effect of the isolated factors (Spacing and Days of evaluation) (Table 4).

The DM content showed no difference with the spacing treatments. However, there was a variation in time that evolves as the plants develop. As far as the NDF and ADF, no difference was observed from the spacing treatment as well. However, an upward growth to a maximum point, followed by a slight decrease was observed only from the days.

Table 4 Analysis of variance, mean estimates, and standard error of the bromatological composition of the Edible Fraction (EF) of *Cratylia* in the 2 and 3 m spacing between rows, regardless of the days of evaluation

Variables	Spacing		SE ⁵	P-Value ⁶		
	2	3		Spac.	Days	SxD ⁷
CP (%) ¹	21,51 a	20,85 b	2,10*10 ⁻³	0,03	1,47*10 ⁻²¹	0,07
NDF (%) ²	56,94 a	57,29 a	3,06*10 ⁻³	0,42	3,49*10 ⁻²²	0,42
ADF (%) ³	40,12 a	40,41 a	2,99*10 ⁻³	0,67	3,97*10 ⁻¹⁰	0,78
DM (%) ⁴	29,62 a	29,61 a	0,33	0,98	3,53*10 ⁻²⁷	0,13

Means followed by different letters are statistically different according to Fisher's test (P<0.05).

¹ Mean of Crude Protein contents (%)

² Mean of Neutral Detergent Fiber (%)

³ Mean of Acid Detergent Fiber (%)

⁴ Mean of Dry Matter (%)

⁵ Mean Standard Error

⁶ P-Value

⁷ Interaction between Spacing and Days

Discussion

The ideal temperature range for plant development and basal temperatures, are not known yet. However, low average temperatures retard its growth. Regarding rainfall, the amount of rainfall in the period allowed to infer that the growth of *Cratylia* was not slowed down by the lack of soil moisture, which as also stated by Matrangolo et al. (2018).

Regarding the whole plant growth curve, both spacings had a similar response with a very small growth at 21 days (6.41 g DM/plant for 2 m spacing and 9.24 g DM/plant for 3 m spacing). This was followed by an accelerated growth with a growth reduction after 84 days (320.68 g for the 2 m spacing and 434.00 g for the 3 m spacing).

The EF behavior was very similar to that of the whole plant in both spacings. This indicates that during plant development, leaves and young stems appear in similar proportions, which composes the EF. This result indicates that *Cratylia* is a forage capable of maintaining nutritional quality over time, from the standpoint of the proportion between EF and the whole plant.

At 105 days of regrowth, higher values of dry matter production and dry matter production of EF were obtained. The values were 816.15 and 631.37 g for 2 and 3 m spacing; 555.91 and 471.37 g for 2 and 3 m spacing; 43.27 and 38.14 g for 2 and 3 m spacing, respectively. After 105 days, growth ceased or continued very slowly, which indicated a trend of deceleration in growth for the productivity variables.

According to the regression models, the optimal DM accumulation timing occurred on day 86 and 91 for 2 and 3 m spacings, respectively. The productivity values also suggested the following days for harvest, based on the evaluated spacings and the current experimental conditions. The use in later periods represented a decrease in productive efficiency for both planting spacings, especially after the 105 days of regrowth.

The premature harvest of *Cratylia* prior to its optimal harvest time could provide a forage with a greater CP and lower levels of NDF and ADF. However, the harvest of plants before their optimal resting point can weaken plant reserves for a new regrowth, which could compromise the future regrowth and the

perenniality of the plant (Machado Filho et al., 2021).

However, these time intervals may not be fixed, since water availability, climatic conditions, the level of defoliation, fertility and other factors influence the growth rate of forage plants (Lima et al., 2020). In this sense, there are important research that seek to understand the ecophysiology of *Cratylia* with the objective of using its biomass in an optimized way. Understanding how to relate the resting time, the establishment of reserves and the impact on regrowth with successive harvest for different edaphoclimatic conditions can help to increase productive efficiency.

The 3 m spacing between rows produced more biomass individually, regardless of the evaluation period, than the 2 m spacing. This may have occurred due to the greater solar radiation absorbed and the greater space for the development of stems. However, considering the density of plants/ha, the 2 m spacing between rows should result in greater yields per hectare than 3 m spacing. This result also indicates that the intraspecific competition is not being a decisive factor for a decreased production over the 2 m spacing. In the final evaluation period, it was observed that growth rates of the whole plant and the EF were very similar between spacings. Therefore, it was possible to point out that the development of biomass in the final stages of evaluation was not impaired by spacing of the 2 m spacing compared to 3 m spacing based on intraspecific competition.

The *Cratylia* harvest at 105 days showed a productivity of 8.16 t/ha (DM) for and 4.21 t/ha (DM) for 2 and 3 m spacings in each growing cycle, respectively. This also indicates that the 2 m spacing between rows was more efficient, although the individual productivity may be lower, the total area productivity exceeded the yields found over the 2 m spacing. It can then be inferred that the increase in plant density promoted an increase in *Cratylia* productivity. Other studies could investigate what would be the maximum density that the *Cratylia* coexists without a decrease in productivity.

There are difficulties in evaluating the productivity growth curve due to the destructive method of evaluation requiring new plants in each growing cycle (Santos et al., 2021). Productivity was a parameter with a lot of variation between plants, since *Cratylia* does not have cultivars, only ecotypes with high genetic variability. The possibility of evaluating biomass production through technologies that do not require harvesting the plant would allow growth evaluations to be performed in the same individuals drawn for each spacing. It is also important to highlight the need for breeding programs to select plants specifically aimed for use in animal feed. Nowadays, there are technologies to carry out this type of evaluation in pastures (Santos et al., 2021), however, the viability in forage shrubs such as *Cratylia* is still unknown. A possibility would be to estimate biomass by measuring height, followed by counting the number of stems, number and size of leaves. Another possibility would be the periodic use of images taken by drones.

Due to the scarcity of research related to *Cratylia* and its optimal herbage accumulation point, the comparisons made are in different conditions of the experiment. Matrangolo et al. (2019) used a fixed time of 90 days to harvest the *Cratylia* as a source of green manure, at the experimental station of Embrapa Maize and Sorghum in Sete Lagoas, Brazil. The authors found a productivity value per plant of 2,600 g with a 4 m spacing between rows and 0.5 m between plants. Panadero et al. (2020) evaluated the potential of *Cratylia* as a forage bank in tropical Colombia. Performing harvests at 45 days of regrowth, they found values of 157 g per plant using a dense culture with a 1 m spacing between rows and 1 m between plants. The estimated productivity for 45 days in this work in both spacings were higher than the values found by

Panadero et al. (2020). The productivity values found by Panadero et al. (2020) and Matrangolo et al. (2018) indicate that the use of *Cratylia* at 45 days of regrowth represents a reduction in the productive potential.

Due to the limited availability of information regarding the development of the plant's physiology for its use, manuscripts of literature review could contribute to the use efficiency of *Cratylia*. Arguello-Rangel et al. (2019) reviewed the use of *Cratylia* for animal feed and evaluated several parameters. The authors found harvest times ranging from 60 to 550 days, however it was not pointed out which would be the best periods for using the plant. Depending on the results obtained, it can be estimated that regrowth periods longer than 105 days may decrease the productive efficiency of *Cratylia*. Furthermore, at very advanced ages, the branches become thick and very lignified (Matrangolo et al., 2018), which could reduce the nutritional potential. It is important to emphasize the difficulty of carrying out a review for a species whose works are scarce and are spread across different conditions in Latin America. This fact results in a lack of standardization of information to arrive at consistent conclusions.

The 2 m spacing resulted in plants with greater CP contents in the EF between the evaluation days. This result may have occurred due to the greater shading of the leaves caused by greater density. There are no studies evaluating the effect of shading on the productivity and nutritional composition of *Cratylia*, but similar results are found in tropical grasses and legumes (Faria et al., 2018; Lista et al., 2019).

It can be said that CP levels combined with DM production are decisive when investigating the optimal herbage accumulation for harvest or grazing. In this sense, in addition to greater productivity, the 2 m spacing between rows provided a greater CP level regardless of the day of evaluation. At 105 days of regrowth, CP values for 2 and 3 m spacing were 20.26 and 18.97% in EF, respectively.

However, the regression estimates provided different models for the spacings, while the model for the 2 m spacing was linear. Thus, CP decreased over time and the minimum value was found at day 126. The 3 m spacing had a quadratic model, and CP contents reached a minimum at day 113 followed by an increase. This may have occurred due to the increased shading of the plant itself at the basilar leaves and by adjacent plants, due to the size and architecture of the plants.

The protein contents found for both spacings were similar to those found in literature. Braga et al. (2018) when evaluating the protein contents extracted from the leaves of *Cratylia* at 55 and 75 days of regrowth, found values of 27.62 and 22.42%, respectively. The estimated CP values for 2 and 3 m spacings, respectively for 55 days are 22.67 and 21.36%, and for 75 days are 21.43 and 19.72%. It was also possible to observe the drop in protein levels over the two evaluated days. It should be noted that CP evaluations occurred exclusively for the leaves, which leads to greater protein contents than the evaluation of the entire EF.

Panadero et al. (2020) evaluated the CP content of *Cratylia* based on different parts of the plant. The results were 17.5, 15.5 and 11.5% for leaves, whole plant, and stems, respectively. This difference may have occurred due to soil fertility conditions, climate differences and genetic material. It should be noted that the authors worked with evaluations at 45 days of regrowth. In the same period, the estimated protein levels for FC are 23.29 and 22.43% for 2 and 3 m spacings, respectively. This difference suggest that protein contents suffer interference from soil fertility, in addition to being sensitive to spacing.

The CP values found in the current experiment are greater than those of tropical grasses used for livestock in central Brazil. Cassimiro et al. (2020) when evaluating the response of Marandu grass

(*Brachiaria brizantha*) to different sources and doses of fertilization found maximum values of 11.30% CP in the entire plant. Barbero et al. (2021) when preparing a review of Brazilian livestock grazing and main species, found maximum CP values of 15% for grasses in the rainy season. The CP levels were also greater than those found in other tropical legumes commonly used in cattle ranching. List et al. (2019) found CP values of 16.64% for perennial soybean (*Neonotonia wightii* Lackey) grown in full sun during the rainy season. Albores-Moreno et al. (2020) found CP values of 14.24% for leucaena (*Leucaena leucocephala*), another tropical shrub legume used in cattle farming and widely disseminated in Brazil.

According to Teixeira et al. (2019), about 90% of the concentrate feeds marketed in Brazil and used for dairy cows, have CP contents between 20 and 22%. These values are close to those found for Cratylia in this experiment and in the literature (Canul-Solis et al., 2020). An important advance in the use of Cratylia would be studies that sought to evaluate the digestibility of Cratylia and the effects on animal performance.

The increase in daily CP intake that Cratylia can promote in tropical grasses can result in increased performance and a reduction in GHG emissions (Canul-Solis et al., 2020; Albores-Moreno et al., 2020). In addition, the Cratylia maintains its green leaves throughout the dry period in several evaluated conditions (Matrangolo et al., 2018; Mattar, 2018). This property of Cratylia is a differential for the supply of high-quality forage, in the period when grasses decrease production and, mainly, lose nutritional quality (Barbero et al., 2021). The use of Cratylia as forage can reduce the negative effects associated with water stress periods and increase livestock efficiency.

Panadero et al. (2020) when evaluating the bromatological composition of Cratylia with 45 days of regrowth in a Colombian degraded soil, found substantially higher fiber values. Evaluating portions of leaves, entire plant and stems, respectively found 72.4, 70.3 and 82.8% for NDF and 45.6, 50.5 and 65.5% for ADF. The values found for all portions are greater than the fibrous portions found for 2 and 3 m spacings in the current research. Estimating the fibrous portions of EF for 45 days at 2 and 3 m spacings, respectively, 54.61 and 55.21% of NDF and 39.30 and 39.36% for ADF. Taking into account the values found for CP and the fibrous portions, it can be said that the current research showed a higher quality forage than Panadero et al. (2020).

It is also possible to find an increase in the fibrous portion over the research of Braga et al. (2018). When evaluating the forage composition of Cratylia in the eastern region of the Brazilian Amazon at 55 and 75 days of regrowth, they found 63.3 and 65.92% for NDF and 55.61 and 56.59 for ADF, respectively. The estimated values for 2 and 3 m spacings at 55 days were 56.08 and 56.85% for NDF and 40.15 and 40.21% for ADF, respectively. While the estimated values for 75 days were 58.41 and 59.29% for FDN and 41.29 and 41.42% for FDA, respectively. The NDF values estimated in this work were lower than those found by Braga et al. (2018), while the ADF values were close to the estimated ones. The NDF and ADF values found had a very similar behavior between the two variables and spacings. However, there was an unusual decrease on fibrous portion after the maximum herbage accumulation. This opposed to a progressive increase in fiber content with time, which is often found in literature (Braga et al., 2018). That might be explained by the methodology used: the EF was always evaluated, which was made up of leaves and branches smaller than 5 mm. This decrease in the fibrous portion in the EF may be due to shading of the leaves caused by the increase in plant size.

Braga et al. (2018) indicates that the Cratylia in both regrowth periods obtained adequate in-situ digestibility for use as a protein bank in sheep diets. The spacing used in the current experiment opens up the possibility of seeding Cratylia with other plants, including grasses. The consortium with grasses allows other forms of use for the Cratylia, such as direct grazing in mixed sward pastures. Matrangolo et al. (2022) obtained good results in the intercrop between Cratylia and Piatã grass (*Brachiaria brizantha*) on the survival of Cratylia plants. Intercropping with other plants of interest such as beans and corn, and the use of Cratylia biomass to animal feeding are other forms of use (Matrangolo et al., 2018). In this sense, research that seeks new technologies for mechanized harvesting of biomass and hay efficiency is important.

Conclusions

For the conditions of the experiment and for the period evaluated, the optimal harvest timing for Cratylia was 86 and 91 days for 2 and 3 m spacing. The use in later periods, especially after 105 days of rest, represented a decrease in the bromatological composition and in productive efficiency. The 2 m spacing between rows was more efficient in terms of productivity and showed higher levels of CP in EF.

Data Availability

The data that support the findings of this study are available on re-quest from the corresponding author.

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