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Research Paper

Influence of plant population density of *Chamaecrista rotundifolia* on its value for hay making in the Eastern Amazon, Brazil

Efecto de la densidad de plantas de Chamaecrista rotundifolia *en su potencial para henificación en la Amazonía Oriental, Brasil*

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Abstract

Chamaecrista rotundifolia is a forage legume little used with Brazilian livestock; however, it has been studied for this purpose for over 40 years in Australia. The aim of this study was to characterize the influence of plant densities of approximately 444,400, 111,100 and 27,800 plants/ha (equivalent to spacings of 0.15 × 0.15; 0.30 × 0.30 and 0.60 × 0.60 m) on quantitative and qualitative parameters of *C. rotundifolia* grown in pure stands as forage under exclusive cropping for hay. While leaf dry matter yields in the first 93 days after planting ranged from 1.48 to 9.32 t DM/ha, declining to 0.71–4.92 t DM/ha in the subsequent 83 days, crude protein concentration of the material was only 7–8 %. Since this species tends to lose leaf during periods of stress, larger paddock studies are needed to determine how well leaf material is retained under conventional hay-making conditions. Optimal stubble height following harvesting should be investigated in an endeavor to increase DM yields at second harvest along with improved survival of plants.

Keywords: Animal nutrition; fodder conservation; forage legumes; plant morphology.

Resumen

Chamaecrista rotundifolia es una leguminosa forrajera poco utilizada con el ganado brasileño; sin embargo, se ha estudiado para este propósito durante más de 40 años en Australia. El objetivo de este estudio fue caracterizar la influencia de densidades de plantas de aproximadamente 444,400, 111,100 y 27,800 plantas/ha (equivalente a espaciados de 0.15 × 0.15; 0.30 × 0.30 y 0.60 × 0.60 m) sobre parámetros cuantitativos y cualitativos de *C. rotundifolia* cultivado en masas puras como forraje bajo cultivos exclusivos para heno. Mientras que el rendimiento de materia seca de las hojas en los primeros 93 días después de la siembra osciló entre 1.48 y 9.32 t MS/ha, posteriormente descendió a 0.71–4.92 t MS/ha en los 83 días posteriores. La concentración de proteína bruta del material fue solo del 7–8 %. Dado que esta especie tiende a perder hojas durante los períodos de estrés, se necesitan estudios más amplios en los potreros para determinar qué tan bien se retiene el material vegetativo en las condiciones convencionales de producción de heno. Se debe investigar la altura óptima del rastrojo después de la cosecha en un esfuerzo por aumentar los rendimientos de MS en la segunda cosecha junto con una mejor supervivencia de las plantas.

Palabras clave: Conservación de forraje; leguminosa forrajera; morfología; nutrición animal.

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Introduction

Legume hay is primarily made from alfalfa, although there are many alternative legumes, mainly in tropical environments. *Chamaecrista rotundifolia* (Pers.) Greene is a legume native to South America (Argentina, Bolivia, Brazil, Colombia, Paraguay, Uruguay and Venezuela), Central America (Costa Rica and Panama) and North America (Mexico) and is naturalized in countries such as the USA and in Africa, in addition to being studied and used as forage for more than 40 years in Australia. It is a weak perennial or self-regenerating annual (in areas with heavy frost or a long dry season) with prostrate growth when young; when older its floral branches tend to die. The main stem is erect to about 1 m high (rarely to 2.5 m) and laterals are ascendant with stems of 0.45–1.1 m long (Cook et al. 2020). According to Strickland et al. (1985), Cruz (1996) and Abreu et al. (2020) this is an interesting species with forage potential, which has stimulated research into cropping techniques and use for animal feed.

Under high-density pure stands (0.25 × 0.25 m) in tropical areas, *C. rotundifolia* yields of up to 22.4 t DM/ha with protein concentration of 8.0 % at 133 days after planting have been obtained (Abreu et al. 2020). These data suggest that the species could be utilized for hay production via mechanical harvesting. Lopes (2001), reporting on the species's potential for green manure, recommended planting of *C. rotundifolia* under spacings of 0.5 and 1.0 m for biomass and seed production, respectively.

High nutrient composition of legumes makes them ideal for hay production, while characteristics such as ease of dehydration and leaf retention are also highly desirable. Several factors which are intrinsic to forage plants, e.g. cuticle thickness, diameter and length of stem and leaf:stem ratio, can have impacts on the drying process (Neres et al. 2010).

There are information gaps in cropping recommendations for legumes, mainly in terms of exclusive cropping for hay production. While growing species in pure stands facilitates hay production, plant density can affect forage yield and quality as a consequence of plant competition. Density effects may vary among species; for example, the biomass of individual plants of *Arachis pintoi* cultivar 'Belmonte' was reduced dramatically when planted at high density, but the total yield per unit area increased (Mamédio et al. 2020).

We hypothesized that a similar situation would occur with *C. rotundifolia* and designed this study to quantify the influence of plant density on quantitative and qualitative parameters of this legume in pure stands.

Materials and Methods

The study was carried out on the experimental area at Universidade Federal Rural da Amazônia (UFRA), Campus Paragominas, Pará state, Brazil (2°59′26″ S, 47°24′24″ W), between December 2018 and June 2019. The climate of Koppen's Aw zone is characterized by a distinct rainy season and a 6-month dry season (Bastos et al. 1993). Average temperature during the experimental period was 30.8 ºC with total precipitation of 2,082 mm (Figure 1).

The soil is Typic Hapludox (uniform, deep, clayey) and physical analysis of the soil indicated composition of 77, 12.6 and 10 % of clay, silt and sand, respectively. Chemical analyses showed: pH ($CaCl_2$) – 5.1; P resin (phosphorus) – 19 mg/dm^3; K (potassium) – 3.7 mmolc/dm^3; Ca (calcium) – 14 mmolc/dm^3; Mg (magnesium) – 5 mmolc/dm^3; H+Al (potential acidity) – 20 mmolc/dm^3; and V (base saturation) – 54 %. While *C. rotundifolia* is well adapted to soils with low pH and base saturation, 1.3 t limestone/ha with total relative neutralizing power of 88 % was applied in September 2018, according to the base saturation method (V%) to elevate the base saturation of soil to 70 % (Hohnwald et al. 2005).

Seeds from native Brazilian plants of *Chaemacrista rotundifolia* (Pers.) Greene var. rotundifolia were

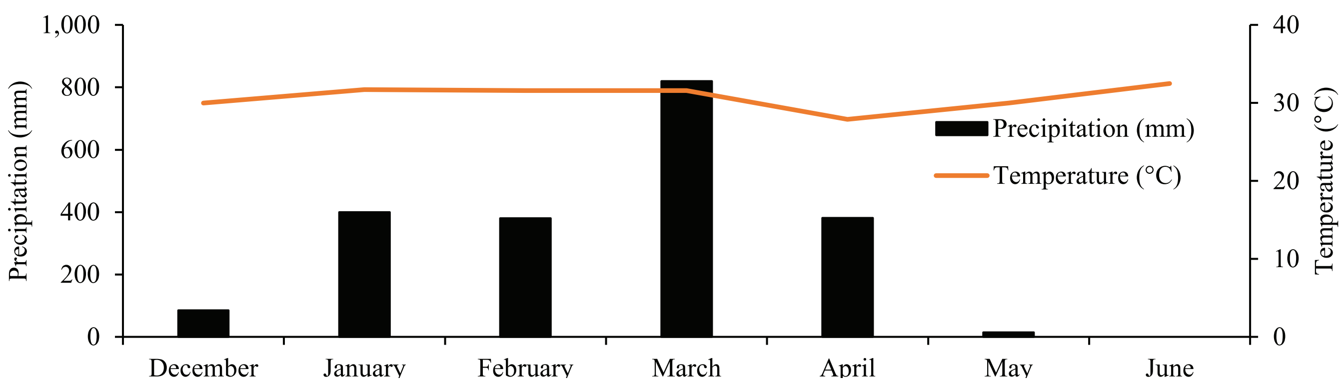

Figure 1. Monthly precipitation and mean temperature during the experimental period (INMET 2019).

collected manually and treated with hot water at 80 ºC for 30 sec followed by immersion in water at room temperature (30 °C) for 12 hours to break seed dormancy (Gomes et al. 2021). Seedlings of *Chamaecrista* were then produced on trays with substrate in a greenhouse and the experiment was established by transplanting seedlings on 28 December 2018.

Weeds were controlled manually during the experimental period. Fertilizers were applied according to Hohnwald et al. (2005) with 8.9 kg P/ha as simple superphosphate at transplanting of seedlings and 7.5 kg N/ha as urea and 6.7 kg K/ha as potassium chloride after 7 days.

A randomized complete block design was used. Treatments comprised 3 planting densities (approx. 444,400, 111,100 and 27,800 plants/ha; treated as high, intermediate and low densities), equivalent to plant spacings of 0.15 × 0.15, 0.30 × 0.30 and 0.60 × 0.60 m, respectively, with 7 replications. Plot size was 1.8 × 1.8 m. In February and March (before the canopy was closed) leaf necrosis was observed in young plants, possibly caused by incidence of fungi favored by high rainfall and low soil drainage, independently of treatment. We controlled this problem by spraying plants with a solution of 2.3 kg copper oxide in 300 L water per hectare, while at the second harvest (in the dry season) no symptoms of necrosis were observed.

Harvests were carried out based on visual assessment, aiming at harvesting with a balance between forage production and quality, as well as allowing natural reseeding, i.e. plants had more than 50 % of open pods (Abreu et al. 2020). The first harvest was performed on 31 March 2019, i.e. 93 days after transplanting of seedlings, while the second occurred after a further 83 days on 22 June 2019 at the beginning of the dry season. At sampling for each harvest 3 whole plants in the central area (1 m^2) from each plot were selected, although the branches were spread over a larger area. The 3 plants were harvested at 5 cm from ground level and harvested material was separated manually into stem and leaf. Plant components were weighed and diameter (StD) and length (StL) of stems were recorded. Average diameter was determined by taking measurements at 3 points along the main stems using a digital caliper, while average length was measured on main stems using a tape.

Plant components were dried in a forced-air circulation oven at 65 ºC for 72 hours and weighed to determine dry matter yield. Samples from the first harvest were ground in a Willey mill prior to chemical analysis. Chemical analyses of plant components were performed as follows: dry matter (DM) by oven-drying at 105 ºC; crude protein (CP) by microKjeldahl method according to AOAC (2011); and neutral detergent fiber and acid detergent fiber using the autoclave method described in Detmann et al. (2012).

Data were organized using Excel® and were tested for normality by the Shapiro-Wilk technique before statistical analysis and any variable that failed to follow a normal distribution was transformed through the procedure of BOX-COX from package “fpp” on software R. All data were analyzed by ANOVA using the packages “expdoes.pt”, “emmeans” and “agricolae” from software R as a randomized complete block design with 3 planting densities and 2 harvest dates as fixed factors. To test for significance, variables were compared by Tukey test at P<0.05 by command “pwpm”.

Results

Significant interactions between plant density and harvest date for DM production of *C. rotundifolia* leaves were recorded (Table 1). Leaf yield per plant decreased and leaf yield/ha increased progressively as plant density increased (P<0.05; Table 1). In general, leaf yields at the first harvest were superior to those at the second harvest (Table 1).

Table 1. Effects of plant spacing and harvest on leaf dry matter production (LDM) of *Chamaecrista rotundifolia*.

	LDM (g/plant)		LDM (t/ha)	
Plant density[1]	Harvest 1	Harvest 2	Harvest 1	Harvest 2
0.60 × 0.60 m	53.5Aa	25.6Ba	1.48Ac	0.71Ab
0.30 × 0.30 m	52.6Aa	22.2Bab	5.65Ab	2.39Bb
0.15 × 0.15 m	21.0Ab	11.1Bb	9.32Aa	4.92Ba
P value	<0.001		0.0085	
CV (%)	26.9		35.9	

[1]Identified as low, intermediate and high density, respectively. Means within columns followed by same lower-case letters and within rows and parameters followed by same upper-case letters do not differ significantly (P>0.05) by Tukey test.

There was no significant interaction between plant spacing and harvest date for production of *C. rotundifolia* stem, but plant density influenced yields (Table 2). Stem production per plant was greater at the 2 wider plant spacings than at the narrow spacing, while production per ha increased as density increased (P<0.05; Table 2). Stem yields were independent of harvest date (P = 0.08).

There was no significant interaction between plant density and harvest date for leaf:stem ratio of plants, but plant density (P = 0.0046) and harvest date (P<0.001) each had significant effects (Table 3). The highest leaf:stem ratios were found at the low and intermediate plant densities, while leaf:stem ratio at the first harvest was greater than at the second harvest (Table 3).

Table 2. Effects of plant density and harvest date on stem dry matter (SDM) production of *Chamaecrista rotundifolia*.

	SDM (g/plant)	SDM (t/ha)
Plant density[1]		
0.60 × 0.60 m	42.0a	1.18c
0.30 × 0.30 m	39.2a	4.39b
0.15 × 0.15 m	25.3b	11.29a
P value	<0.001	<0.001
CV (%)	6.01	11.37
Harvest		
Harvest 1	0.68a	4.19a
Harvest 2	0.66a	3.57a
P value	0.0800	0.0810
CV (%)	6.01	11.37

[1]Identified as low, intermediate and high density, respectively. Means within columns followed by same letters do not differ significantly (P>0.05) by Tukey's test.

Table 3. Effects of plant density and harvest date on leaf:stem ratio of *Chamaecrista rotundifolia*.

Plant density[1]	Leaf:stem ratio
0.60 × 0.60 m	0.90a
0.30 × 0.30 m	0.89a
0.15 × 0.15 m	0.61b
P value	0.0046
CV (%)	29.8
Harvest	
Harvest 1	1.04a
Harvest 2	0.56b
P value	<0.001
CV (%)	29.8

[1]Identified as low, intermediate and high density, respectively. Means within columns and parameters followed by same letters do not differ significantly (P>0.05) by Tukey's test.

There was no interaction between plant density and harvest date for total DM production per plant or per hectare (Table 4). However, both density and harvest date influenced total DM production. Low and intermediate plant densities presented higher yields per plant than high plant density (P<0.001); on the other hand, production per hectare was directly related to plant density (P<0.001), i.e. highest production occurred at the highest plant density and lowest production at the lowest plant density. Highest yields per plant and per hectare occurred at the first harvest (Table 4).

There was no significant interaction between plant density and harvest date for stem length (StL) (P>0.05), but intermediate plant density showed greater stem length than high plant density (P = 0.0065; Table 5). However, a significant interaction between plant density and harvest date occurred for stem diameter with no effect of plant density on stem diameter at the first harvest, while stem diameter at the low plant density exceeded that at the high density at the second harvest (P = 0.0046).

Chemical analyses of forage from the first harvest showed that plant density affected all parameters, except for CP and H concentrations in stem (Table 6). In general, CP, NDF, ADF and H concentrations in leaf and NDF and ADF concentrations in stem were lowest (P<0.05) at the high plant density.

Table 4. Total DM production (leaf plus stem) per plant and per hectare.

Plant density[1]	Total production (g/plant)	Total production (t/ha)
0.60 × 0.60 m	80.0a	2.32c
0.30 × 0.30 m	73.8a	8.68b
0.15 × 0.15 m	40.9b	19.56a
P value	<0.001	<0.001
CV (%)	5.9	39.7
Harvest		
Harvest 1	76.6a	12.02a
Harvest 2	50.6b	8.36b
P value	<0.001	0.0064
CV (%)	5.9	39.7

[1]Identified as low, intermediate and high density, respectively. Means within columns and parameters followed by same letters do not differ significantly (P>0.05) by Tukey's test.

Table 5. Effects of plant density and harvest date on length (StL) and diameter (StD) of stems of *C. rotundifolia*.

Plant density[1]	StL (cm)	StD (mm)	
		Harvest 1	Harvest 2
0.60 × 0.60 m	73.1ba	2.67Aa	2.31Ba
0.30 × 0.30 m	76.9a	2.87Aa	2.04Bab
0.15 × 0.15 m	63.3b	2.91Aa	1.95Bb
P value	0.0065	P value	0.0046
CV (%)	19.0	CV (%)	18.1

[1]Identified as low, intermediate and high density, respectively. Means within columns followed by the same lower-case letter and within rows for StD followed by the same upper-case letter do not differ significantly (P>0.05) by Tukey test.

Table 6. Effects of plant density on crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose (H) concentrations in leaf and stem of *Chamaecrista rotundifolia*.

Plant density[1]	Leaf				Stem			
	CP	NDF	ADF	H	CP	NDF	ADF	H
0.60 × 0.60 m	10.3a	49.4a	28.4ab	21.1a	6.5a	75.7a	58.6b	16.2a
0.30 × 0.30 m	9.6ab	51.9a	30.5a	22.6a	6.2a	77.5a	61.1a	16.4a
0.15 × 0.15 m	8.6b	42.2b	26.0b	16.2b	5.6a	68.9b	52.6c	16.3a
P value	0.0298	<0.001	<0.001	<0.001	0.2439	<0.001	<0.001	0.9571
CV (%)	12.9	10.5	10.5	16.2	12.9	6.1	7.3	6.7

[1]Identified as low, intermediate and high density, respectively.
Means within columns followed by same letters do not differ significantly at P<0.05 by the Tukey test.

Discussion

This study has produced some valuable information on growth of *C. rotundifolia* when planted at different densities in pure stands in Brazil. These data provide an indication of likely yield and quality of this species in the particular environment. *C. rotundifolia* showed high potential for leaf production at the first harvest, mainly at the high plant density (Table 1), with markedly lower production (30 % lower) at the second harvest. The reduced yields at the second harvest could be a function of this growth period extending into the beginning of the dry season, combined with forage being harvested at near ground level at the first harvest. Total production (Table 4) of the intermediate treatment (0.3 × 0.3 m) was similar to that of Abreu et al. (2020) at similar plant density (0.25 × 0.25 m), although only one harvest was performed at 133 days after transplanting seedlings.

Virtually no rain was registered after 30 April. Cruz et al. (1999) evaluated accession CIAT 7792 and found no difference in DM production during dry and wet periods, which are characteristic of the Amazon climate. These authors found annual DM yields of 3.55 and 3.39 t/ha for leaf and stem, respectively, in plants spaced at 0.50 m. The magnitude of difference between the 2 studies can be justified by the different soil types, since we cultivated *C. rotundifolia* on a clay soil with N:P:K fertilizer application, while Cruz et al. (1999) evaluated *C. rotundifolia* in sandy soil without fertilizer, and used a different accession and harvest height (30 cm above ground).

Harvesting plants at 5 cm from ground level resulted in long recovery periods following harvesting. It is suggested that harvesting this species near ground level should be avoided to prevent low yields at subsequent harvests. In addition, higher harvest heights should be investigated, since Lopes (2001), in similar climatic conditions, but in sandy soil, obtained up to 6 harvests per year with intervals between harvests of 56 days and cutting at 30 cm above the soil surface. Yields reached 17 t DM/ha from a single harvest at 6 months of age or a total yield of 25 t DM/ha with 3 harvests at 4, 7 and 14 months of age.

Although *C. rotundifolia* is characterized as a prostrate subshrub or small shrub, plant density and harvesting close to ground level can modify the morphological composition of plants, mainly due to competition for light and nutrients or reduction in amount of photosynthetic tissue, respectively. Different planting densities influenced diameter and length of stem, which can be related to height and density of shrubs, and the area occupied by each plant, although these characteristics were not evaluated.

As a result of the drastic cut, regrowth rates were slow and intervals between harvests increased, as also observed in *Arachis pintoi* by Alonzo et al. (2017). Besides, in dry weather, leaves were smaller and presented a different shape (Figure 2). According to Cook et al. (2020) this species is reasonably drought-tolerant with plants forming rosettes under heavier grazing, but leaves often turn red and drop, if plants are left ungrazed and tall during dry conditions. This phenomenon was observed in forage produced during the regrowth period prior to Harvest 2, probably in response to the drop in soil moisture levels, consequently resulting in lower DM production. This is corroborated by values for leaf:stem ratio under the effect of harvest (P = 0.0046) (Table 3) with a higher ratio at the first harvest. Leaf:stem ratio (Table 3) is a plant characteristic with a significant influence on forage drying rates (Neres et al. 2010) and animal intake of forage, so is an important attribute to be considered.

Figure 2. Branch with common, rounded leaves found at first harvest (A) and branch with modified, also smaller leaves from the second harvest (B) of *Chamaecrista rotundifolia* (leaves in B were not diseased).

Estimated DM production per unit area considering both leaves and stems (Tables 1, 2 and 4) increased as plant density increased, i.e. highest at the narrow plant spacing/high plant density at the first harvest. This dynamic is a function of the greater number of plants being able to utilize the available resources, i.e. water, light and nutrients. However, during the second growing season, water availability, in particular, may have become a limiting factor for maximum growth, particularly of leaves, in addition to the limited amount of photosynthetic material available (Table 1). It was evident that high density resulted in a decrease in individual components (leaves and stems) of the plants (see individual DM production in Tables 1 and 2) according to the well-known deleterious effect of intra-specific competition. Interestingly, Vieira et al. (2016) described *C. rotundifolia* plants as extremely sensitive to drought conditions, which can significantly reduce biomass production, sometimes even resulting in plant death. On the other hand, Partridge and Wright (1992) described the Australian cultivar 'Wynn' of this species as having excellent adaptive capacity and being competitive in high plant densities, even when intercropped with other species, such as grasses. However, the cultivar used in this study is native to Brazil at about 23° S according to Cruz et al. (1999); in the studies of Lopes (2001) and Abreu et al. (2020) it demonstrated a desirable adaptation at latitudes about 1–3° S, similar to 'Wynn' also showing susceptibility to drought.

Lopes (2001) recommended planting this species at spacings of 0.5 and 1.0 m for the formation of pure stands. Our study showed closer plant spacings can result in high DM yields, e.g. at plant spacings between 0.15 × 0.15 and 0.3 × 0.3 m (Tables 1 and 2). High DM yields of forage are particularly desirable when harvesting forage for silage or hay production or cut-and-carry feeding, mainly due to reduction in processing costs per tonne.

Lopes (2001) and Camarão et al. (2008) described this species as being well adapted to sandy soils, and even infertile soils in the Amazon. Our results indicated that excellent yields can be reached on predominantly clay soils as well, corroborating the findings of Abreu et al. (2020) in the same edaphoclimatic environment. However, CP concentration varied from 8.6 to 10.3 % for leaves and 5.6 to 6.5 % for stems, which are low values for a leguminous plant. These values are lower than those found by Cruz et al. (1999) of 16.0 and 18.6 % for leaves and 5.5 and 9.1 % for stems in the dry and rainy seasons, respectively, in a sandy Yellow Latosol, although these authors obtained forage yields of only 4.1 t DM/ha in the wet season and 2.84 t DM/ha in the dry season.

The dynamic of production versus quality in both studies emphasizes the importance of considering both quantity and quality of a species when determining harvest frequency. Overall CP concentration of forage compared favorably with the 7 % CP, below which animal intake can be compromised (Berchielli et al. 2006). While higher plant densities resulted in increasing DM yields, forage produced under wider spacing showed better nutritional quality as leaf:stem ratio was superior to those at narrower spacings (Table 2). Although plant spacings between 0.15 × 0.15 m and 0.30 × 0.30 m seem to be viable for hay or forage production in tropical areas to maximize DM yield, the forage produced would be of lesser quality than at wider spacing. However, the increased yields would far outweigh any reduction in quality.

Climatic conditions of the Amazon of high rainfall and temperature along with poor soil drainage can provide a favorable environment for fungal growth. Cruz (1996) observed that 6 accessions of *C. rotundifolia* (BR 000183, 000205, 000191, 000264, 000272 and 000256) were susceptible to the fungi *Phomopsis subcircinata* and *Rhizoctonia solani*, although with little resultant damage. In that study the author worked with a harvest height of 20 cm, but recommended harvesting above 30 cm at 56-day intervals, as harvests at 20 cm at the same frequency caused the death of plants. This is corroborated by Cruz et al. (1999) and Lopes (2001).

Certainly, *C. rotundifolia* is an option for improving livestock production in the Amazon, corroborating the finding of Camarão et al. (2008), but feeding studies would be needed to confirm acceptance by animals. Partridge and Wright (1992) showed improved growth of steers under grazing in Queensland, Australia, when this legume was sown into native grass pastures. One would expect a similar result under conditions in the Amazon.

Pure stands of *C. rotundifolia* certainly showed potential for production of forage in the Amazon under high planting densities. However, attempting to harvest the legume for hay might not be so successful, since this species is recognized as shedding leaves under stress, and poor leaf retention during the hay-making process may be an issue. Additional studies on optimal management strategies, including reducing fungal attacks, persistence under repeated harvests (it is only a weak perennial), acceptance by stock, digestibility, economic factors, soil influences and mechanical methods for planting and harvesting seed and harvesting forage are needed.

Conclusions

Chamaecrista rotundifolia showed potential for forage production in the soil of the experimental site in pure stands at high plant densities, producing yields of up to 14 t leaf DM/ha in 6 months. However, further studies are needed to determine how resilient plants are under repeated harvests, how well forage retains leaf during the hay-making process, how severe are fungal attacks during the rainy season, how irrigation at the beginning of the dry season may impede the decline in production at this time and how productive it can be on different soil textures.

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