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BIOLOGY

Herbaceous forage legumes with diverse structural traits can display similar productive responses under different harvest frequencies

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ABSTRACT: One of the main issues related to the management of forage legumes is the fact that these plants can be found in a broad diversity of morphology, structures and growth habits. This study evaluated the structural and productive characteristics of forage legumes with different growth habits [*Clitoria ternatea* L.; *Desmanthus pernambucanus* (L.) Thellung; and *Stylosanthes* spp. cv. Campo Grande], in response to two harvesting frequencies (60 and 90 days). The experimental design was completely randomized in blocks, with a split-plot scheme and four replications (n=4). The type of forage legume influenced the number of secondary branches, the number of leaves per branch, leaf area index (LAI), light interception (LI), percentage of leaves and stems (%), leaf/stem ratio, and pod production ($P<0.05$). *D. pernambucanus* had the lowest LI and LAI ($P < 0.05$). *Stylosanthes* spp. cv. Campo Grande and *C. ternatea* showed a greater leaf percentage and leaf/stem ratio ($P < 0.05$). There was an effect of the harvesting frequency on the percentage of stems, leaves, pod production, and total production ($P < 0.05$). Longer harvesting frequencies affected differently these legumes, especially the accumulation of stems in *D. pernambucanus*, which displayed an intense increase after 60 days of growth. The choice of the best harvesting frequency for these legumes evaluated should consider the proposal of the usage. In our study, it was shown that harvesting at 60 days did not affect the total forage mass accumulated, which possibly is the most advantageous choice.
Key words: cutting, forage management, plant growth, plant structure, production.

Leguminosas forrageiras herbáceas com características estruturais diversas podem exibir respostas produtivas semelhantes sob diferentes frequências de colheita

RESUMO: Uma das principais questões relacionadas ao manejo de leguminosas forrageiras é o fato de que essas plantas podem ser encontradas em uma ampla diversidade de morfológica, estruturais e de hábitos de crescimento. O objetivo deste estudo foi avaliar características morfológicas, estruturais e produtivas de leguminosas forrageiras com diferentes hábitos de crescimento [Clitoria ternatea L.; Desmanthus pernambucanus (L.) Thellung; e Stylosanthes spp. cv. Campo Grande], em resposta a duas frequências de colheita (60 e 90 dias). O delineamento experimental foi inteiramente casualizado em blocos, com esquema de parcelas subdivididas e quatro repetições (n=4). O tipo de leguminosa forrageira influenciou no número de ramos secundários, número de folhas por ramo, índice de área foliar (IAF), interceptação luminosa (IL), porcentagem de folhas e colmos (%), razão folha/colmo e produção de vagens ($P<0,05$). D. pernambucanus apresentou os menores IL e IAF ($P<0,05$). Stylosanthes spp. cv. Campo Grande e C. ternatea apresentaram maior porcentagem de folhas e relação folha/colmo ($P<0,05$). Houve efeito da frequência de colheita na porcentagem de produção de hastes, folhas e vagens e na produção total ($P<0,05$). Frequências de colheita mais longas afetaram de forma diferente as leguminosas forrageiras avaliadas, principalmente o acúmulo de caules em D. pernambucanus, que apresentou aumento intenso após 60 dias de crescimento. A escolha da melhor frequência de colheita para essas leguminosas avaliadas deve levar em consideração a sua forma de uso. No presente estudo, foi demonstrado que a colheita aos 60 dias não afetou a massa forragem total acumulada, o que possivelmente é a escolha mais vantajosa.
Palavras-chave: colheita, manejo da forragem, crescimento da planta, morfologia e produção.

INTRODUCTION

Cultivating forage legumes can add many benefits to pasture-based livestock systems. Due to their capacity of associating with bacteria that can fix atmospheric nitrogen (N2), the presence of forage legumes in the pastures can increase the quantity and the availability of N in the soil-plant-animal system (SCHULTZE-KRAFT et al., 2018; WANG et al., 2021). The use of legumes in pastures can reduce the demand for the applications of N fertilizers, which is an economic and ecological advantage (JENSEN et al., 2020). In nutritional terms, forage legumes can have great concentrations of crude protein (≈15-25%) (CASTRO-MONTOYA & DICKHOEFER, 2020), which can improve animal diet and performance.

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Despite the benefits of the usage of forage legumes, their cultivation in pasture-based livestock systems is still small compared to the area devoted to the forage grass species (MUIR et al., 2014; PHELAN et al., 2015). Worldwide, efforts have been made to identify and make available potential forage legumes (MUIR et al., 2019; BODDEY et al., 2020); however, there are still many questions related to their management and usage in the different pasture-based systems and environments. One of the main issues related to the management of forage legumes is the fact that these plants can be found in a broad diversity of morphology, structures and growth habits, which require specific management practices for each species. Forage legumes can be found in herbaceous, shrubs, three, and climbers forms, also, they have annual or perennial growth (CASTRO-MONTOYA & DICKHOEFER, 2020). These structural and growth differences make it difficult to settle similar management practices for most of the forage legumes, which require specific management practices for each species (SCHEFFER-BASSO et al., 2002; MUIR et al., 2014).

Forage legumes with different growth habits display different patterns for biomass allocation among leaves, stems, and roots, which implies different management strategies for their harvesting or grazing (SCHEFFER-BASSO et al., 2002). The frequency of defoliation, for example, can directly affect the speed of the legume growth; consequently, the production and quality of the forage. More frequent harvesting can provide a forage with better nutritional composition; however, in the long term, legume productivity and persistence are generally reduced (SILVA et al., 2010). Thus, finding a suitable harvesting frequency for each legume species is essential for reaching a balance between forage production, nutritional quality, and persistence.

This study hypothesizes that forage legumes with different growth habits will respond differently to the same cutting frequency. Thus, the objective of this investigation was to evaluate the structural and productive features of forage legumes with different growth habits under two harvesting frequencies.

MATERIALS AND METHODS

The experiment was carried out in the Sugarcane Experimental Station of the Universidade Federal Rural de Pernambuco (UFRPE), located in the city of Carpina, state of Pernambuco, at an average altitude of 180 m a.s.l. The geographical coordinates were latitude 07º51’03” south and longitude 35º15’17” west. The climate of the region is As’ (dry tropical), according to the classification of Köppen, with the rainy period occurring from May to August. The average temperature and annual precipitations are 24.6 °C and 1,100 mm. The total accumulated rainfall during the experimental period was 1,346 mm. The experiment occurred from January 2019 to March 2020.

The predominant soil in the region is Yellow Argisol, with flat to gently undulating topography. The experimental area was mowed and the soil was prepared with a heavy harrowing. The chemical features of the soil (0-20 cm) were: P (Mehlich-I) = 24 mg.dm-³ pH in water = 5.7; Ca^{+2}= 2.70 cmolc.dm^{-3}; Mg^{+2}= 2.20 cmolc.dm^{-3}; Na^{+}= 0.08 cmolc.dm^{-3}; K^{+}= 0.26 cmolc.dm^{-3}; Al^{+3}= 0.00 cmolc.dm^{-3}; H^{+}+Al^{+3} = 4.20 cmolc.dm^{-3}; CTC= 9.44 cmolc.dm^{-3}; organic matter (OM) = 2.33% and the percentage of bases saturation (V%) = 55.53%.

The experimental treatments consisted of three types of forage legumes *Clitoria ternatea* L., [*Desmanthus pernambucanus* (L.) Thellung.]; and [*Stylosanthes* spp. cv. Campo Grande (*Stylosanthes capitata* Vog. and *Stylosanthes macrocephala* M. B. Ferr. and N. S. Costa)], under two harvesting frequencies 60 and 90 days (Figure 1). Concerning the growth habit, *C. ternatea* L. is classified as a perennial herbaceous climber (creeper) with erect and semi-erect stems (Figure 1a) (SUARNA & WIJAYA 2021) *D. pernambucanus* (L.) Thellung. is a bush legume with woody-like erect stems (VERLOOVE & BORGES, 2018) (Figure 1b). The accession used in this trial was the 7G, reported in CALADO et al. (2016). The cultivar *Stylosanthes* spp. cv. Campo Grande as a mixture of two species, 80% *Stylosanthes capitata* Vog. and 20% *Stylosanthes macrocephala* M. B. Ferr. and N. S. Costa, presents a mixture of erect and semi-erect plants (ALZATE-MARIN et al., 2020) (Figure 1c).

The experimental design was completely randomized in blocks, with a split-plot scheme and four replications (n=4). The forage legumes were allocated to the main plots and the harvesting frequencies (60 and 90 days) were defined as subplots, totaling 24 experimental units. Each plot had a total area of 9 m^2 and each subplot of 4.5 m^2. The distance between blocks was 2 m, and between plots was 1 m. The sampling area was represented by the four central plants in each subplot. All the legumes were initially grown in plastic seedling bags (10 x 15 cm). The seeds of *C. ternatea* L. and *D. pernambucanus* (L.) Thellung were subjected to mechanical scarification with sandpaper. Three seeds were planted per seedling bag. The substrate used was composed of washed sand and clayey soil, in the proportion of

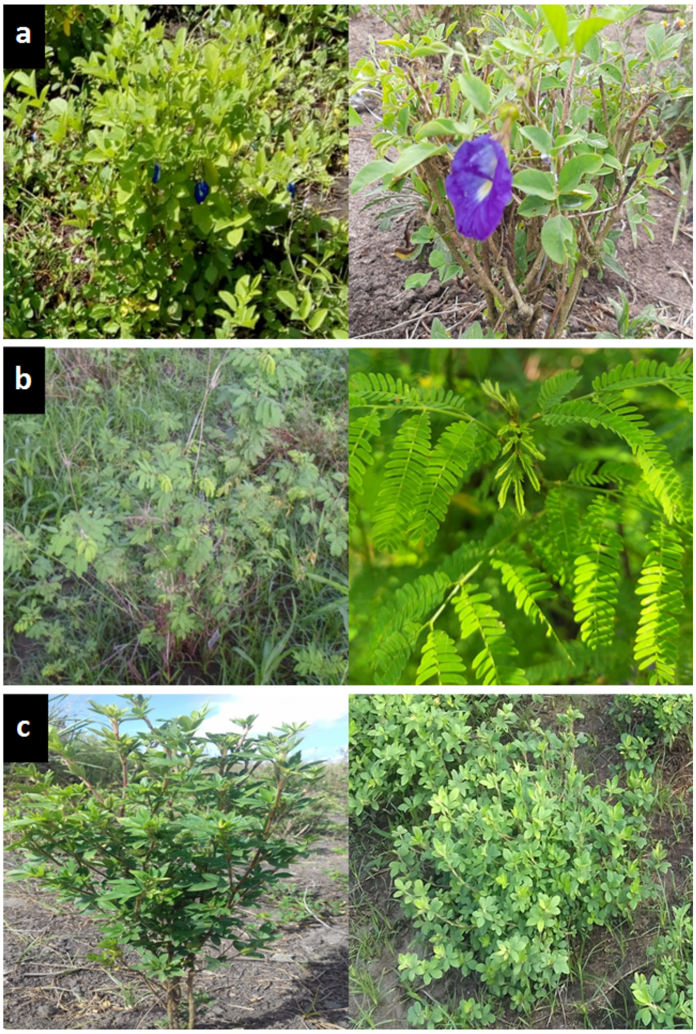

Figure 1 - *Clitoria ternatea* L. (a), *Desmanthus pernambucanus* (L.) Thellung. (b); and *Stylosanthes* spp. cv. Campo Grande (c).

1:3, respectively. The seedlings were grown in a greenhouse, and after 60 days they were taken to the field and transplanted. The seedlings were planted in pits of 20 cm depth, and the space between plants was 0.5 m x 0.5 m. At the time of the planting, 40 kg of P_2O_5 ha^{-1} and 40 kg of K_2O ha^{-1} were applied, within the pits. After 90 days of planting, the plants were subjected to a uniformization cut, at 20 cm height.

The height of the plant was measured using a measuring tape graduated in centimeters (cm), it was considered the distance from the soil level to the highest part of the plant. The width of the plant was measured based on the arithmetic mean of two measurements in the horizontal projection of each plant (taken in two perpendicular axes above the plant), using a measuring tape graduated (cm). The stem diameter and primary branch were measured with a caliper. For the stem, the measurements were performed three centimeters above the soil surface, and for the primary branch, at the base of its insertion into the stem. Branch numbers (primary and secondary), and leaves per branch, were computed based on the mean of their counting. Leaf area index (LAI) and light interception (LI) were estimated before each harvesting (60 and 90 days), using the light meter device Plant Canopy Analyzer LAI-2000 (LICOR®), Lincoln-NE USA. During the measurements of light interception, the first reading was taken above the canopy followed by three readings below the canopy, just above the soil level. The measurements were carried out in the early morning (before 8:00 am) or late afternoon (4:00 to 6:00 pm).

Forage production was determined by harvesting, they were cut leaving 20 cm stubble height. Immediately after cutting, the harvested material was weighed on an electronic digital scale to obtain the total herbage mass in fresh weight. Three plants from each subplot were used as sub-samples, and from these plants, the stems, leaves, and pods were separated, weighed, and dried in a forced-air circulation oven, at 55 ºC for 72 h. Leaf: stem ratio was determined by dividing the dry weight of the leaf fraction by the dry weight of the stem fraction. Forage production (leaves + stems + pods), leaf production, stems production and pod production were estimated in kg of dry matter (DM) ha^{-1}. The proportions of leaves, stems and pods in the forage were estimated. During the experiment, after the uniformization cut, four harvesting were performed in the subplots with a harvesting frequency of 60 days and three in those with 90 days harvesting interval, totaling 270 days. Forage (leaves + stems + pods), leaves, stems, and pods accumulation in 270 days were calculated using the following equation:

$$\text{Forage accumulation (kg DM ha}^{-1}\text{ 270 days)} = \frac{\sum \text{production/harvesting}}{(\text{n}^{\text{o}}\text{ of harvestings x harvesting interval})} \text{ x 270 days}$$

In the ANOVA analysis, forage legume species, harvesting frequencies, and their interactions were considered fixed effects. The blocks were considered random effects. The means were compared using the Tukey test. ANOVA and post-hoc tests were performed using SAS University Edition software, considering a significance level of 5% ($P < 0.05$). To evaluate the relationship between the variables and the factors (legumes and harvesting frequencies), a principal component analysis (PCA) was performed with the variables that showed a significant effect in the ANOVA. The PCA was performed based on the Pearson correlation matrix using the software R Studio®.

RESULTS AND DISCUSSION

Stylosanthes spp. cv. Campo Grande and *C. ternatea* showed greater leaf percentage and leaf: stem ratio ($P < 0.05$), while *D. pernambucanus* had a greater stem percentage ($P < 0.05$) (Table 1). In terms of light interception, *D. pernambucanus* had the lowest LI and LAI ($P < 0.05$). *Stylosanthes* spp. cv. Campo Grande and *C. ternatea* had greater pod production ($P < 0.05$) (Figure 2). The fact *D. pernambucanus*

Table 1 - The number of secondary branches, number of leaves per branch, leaf area index (LAI), light interception (LI) (%), percentage of leaves and stems (%), leaf/stem ratio, and pod production (kg DM ha^{-1}) of different forage legumes, under two harvesting frequencies (60 and 90 days); and. stem diameter (cm), number of secondary branches, proportion of stems (%), leaves production (kg DM ha^{-1}), pod production (kg DM ha^{-1}) and forage production (kg DM ha^{-1}) in function of different harvesting frequencies (60 and 90 days) combining the results of different forage legumes (*Stylosanthes* spp. cv. Campo Grande, *D. pernambucanus*, *C. ternatea*).

	--------------------------------Forage legumes --------------------------------			
	Stylosanthes spp. cv. Campo Grande	*D. pernambucanus*	*C. ternatea*	Standard error of the mean
Number of secondary branches	3.0 a	2.1 b	2.0 b	0.25
Number of leaves per branch	17.0 a	11.0 b	8.0 c	0.70
LAI	1.3 a	0.7 b	1.4 a	0.17
LI	58.0 a	39.0 b	50.0 ab	4.78
Leaves	56 a	42 b	49 ab	0.002
Stems	33 b	51 a	28 b	3.43
Leaf/stem ratio	2.1 a	0.9 b	2.0 a	0.23
Pod production (kg DM ha^{-1})	257 a	62 b	212a	56.8
	---------------------------Harvesting frequency (days)-------------------------			Standard error of the mean
	60		90	
Stem diameter	2.2 b		2.6 a	0.20
Number of secondary branches	8.0 b		9.0 a	0.41
Stems	34 b		40 a	1.19
Leaf production	364 b		546 a	61.96
Pod production	88 b		266 a	46.38
Forage production	708 b		1403 a	160.67

Means followed by the same letter do not differ under the Tukey test ($P > 0.05$). DM= dry matter.
Note: only variables that showed significant results in each factor were showed.

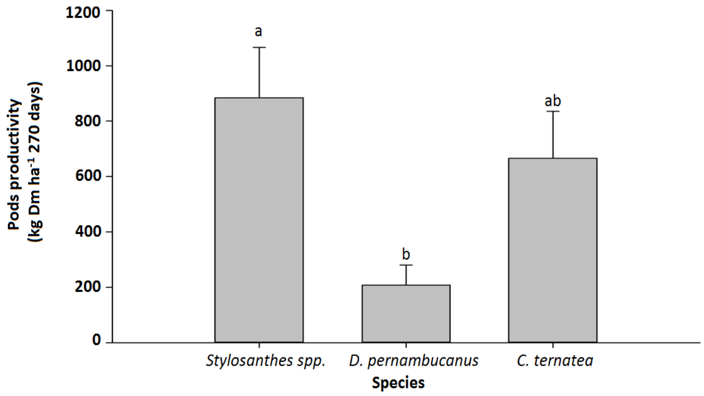

Figure 2 - Pod productivity (kg DM ha^{-1} 270 days) of different forage legumes. Bar means followed by the same letter do not differ under the Tukey test (P > 0.05).

showed lower LAI and LI was possibly related to its type of plant architecture, characterized by longer, and wider spaced branches, with its leaves divided up to the leaflet level (Figure 1b) (VERLOOVE AND BORGES, 2018). The natural lengthening of the stems of *D. pernambucanus* builds a widely open canopy architecture, allowing a great incidence of radiation in the basal and lateral meristems, which might stimulate the growth of new branches (CALADO et al., 2016).

There were interaction effects between the type of forage legume and the harvesting frequencies (Table 2). *D. pernambucanus* was the highest legume

Table 2 - Plant height, plant width, branch diameter, and stem production (kg DM ha^{-1}) of different forage legumes, under two harvesting frequencies (60 and 90 days).

Species	Harvesting frequency (days)	
	60	90
Plant height (cm)		
Stylosanthes spp. cv. Campo Grande	32 aB	33 aB
D. pernambucanus	47 bA	69 aA
C. ternatea	36 aAB	44 aB
Standard error of the mean	3.41	
Plant width (cm)		
Stylosanthes spp. cv. Campo Grande	40 aA	54 aAB
D. pernambucanus	47 bA	69 aA
C. ternatea	34 aA	38 aB
Standard error of the mean	8.49	
Branch diameter (mm)		
Stylosanthes spp. cv. Campo Grande	3.8 aA	4.3 aA
D. pernambucanus	2.9 bA	4.4 aA
C. ternatea	3.9 aA	3.7 aA
Standard error of the mean	0.32	
Stem production (kg DM ha^{-1})		
Stylosanthes spp. cv. Campo Grande	280 aA	435 aB
D. pernambucanus	290 bA	995 aA
C. ternatea	197 aA	384 aB
Standard error of the mean	2.10	

Means followed by the same uppercase letter in the column and lowercase in the lines do not differ in the Tukey test (P > 0.05). DM= dry matter.

evaluated, and different from *Stylosanthes* spp. cv. Campo Grande and *C. ternatea* showed a significant increase in height between 60 to 90 days of harvesting ($P < 0.05$). At 60 days of harvesting, there was no significant difference in plant width between the forage legumes; however, at 90 days *Stylosanthes* spp. cv. Campo Grande and *D. pernambucanus* increased their width, while *C. ternatea* showed no significant difference between its width at 60 and 90 days. The branch diameter did not differ across the species, and only *D. pernambucanus* showed a significant increase in it between 60 and 90 days of growth. At 60 days, stem production was similar between the species ($P > 0.05$); however, at 90 days, there was a pronounced increase in the stems produced by *D. pernambucanus*. There was no significant effect of the type of legume on the accumulated leaf and forage for 270 days ($P < 0.05$). Stem and pod accumulation were increased by extending the harvesting period from 60 to 90 days ($P < 0.05$) (Table 3). There was more influence of the type of legume species (Figure 3a) than harvesting frequency (Figure 3b) on the special distribution and relationship between variables.

Regarding plant responses to harvesting, in general, plants with strong apical dominance can display an intense branching response to the removal of their apical meristem or other expanding tissues with growing points (TAIZ et al., 2017). In our study, only *D. pernambucanus* showed a significant increase in its plant width between 60 to 90 days, this might be linked to a combination of stem elongation, and the horizontal growth stimulated by the development of lateral branches. As a consequence of the removal of the apical meristems, there is a growth stimulation of the lateral buds. In this case, a plant might grow more horizontally, which might increase plant dimension (CALADO et al., 2016). During vegetative development, the axillary meristems, in the same way as the apical meristem, initiate the formation of new leaves, resulting in axillary buds. These buds are dormant, or developed in lateral branches, depending on their position along the axis of the stem. It depends on the stage of the development of the plant and the environmental conditions (TAIZ et al., 2017). For many species, a larger plant width can result in a greater ground cover and light interception, however, for *D. pernambucanus*, LAI and LI values were lesser compared to the other legumes evaluated. This reinforces the hypothesis that an openly branched architecture with leaves composed of small leaflets might reduce the light interception per area

In general, the harvesting period of 90 days contributed to the increase in the accumulation of stems and pods, but not leaves. *D. pernambucanus* showed a great increase in its stem mass produced between 60 to 90 days. As leaves ceased or reduced their growth after 60 days, and there was a substantial increase in stems, it is suggested that *D. pernambucanus* should be harvested earlier than 90 days. Conversely, *Stylosanthes* spp. cv. Campo Grande and *C. ternatea* can be harvested later than 60 days, as their stem accumulation between 60 and 90 days was much lesser, nevertheless, it should consider that forage plants tend to lose their nutritional quality as they age and reach reproductive stages.

During the trial, it was observed that the legumes studied did not enter their reproductive phase until 60 days of growth. However, there were substantial increases in pod production at 90 days. In nutritional terms, it might indicate a lower nutritional value of the forage due to plant maturity (NICODEMO et al., 2015; ERGON et al., 2017). Also, some organs in plants (e.g., pods and fruits), can act as a drain of photoassimilates and nutrients during the reproductive phase (TAIZ et al., 2017). On the other hand, if the objective of the pasture is to produce seeds, it might be advantageous to harvest these legumes later than 60 days. Also, it should be remembered that seed production is a survival strategy, and essential for the

Table 3 - Leaf, stem, pod and forage accumulation (kg DM ha^{-1} 270 days) of different forage legumes, under two harvesting frequencies (60 and 90 days).

Accumulation (kg DM ha^{-1} 270 days)	-------------Harvesting frequency (days)-----------		difference* (%)	Standard error of the mean
	60	90		
Leaf	1637 a	1550 a	-5	219.54
Stem	1150 b	1719 a	50	2.46
Pod	398 b	774 a	95	156.99
Forage	3185 a	4210 a	32	494.56

Means followed by the same letter do not differ in the Tukey test ($P > 0.05$).
*Percentual difference between 60- and 90-days harvesting frequency.

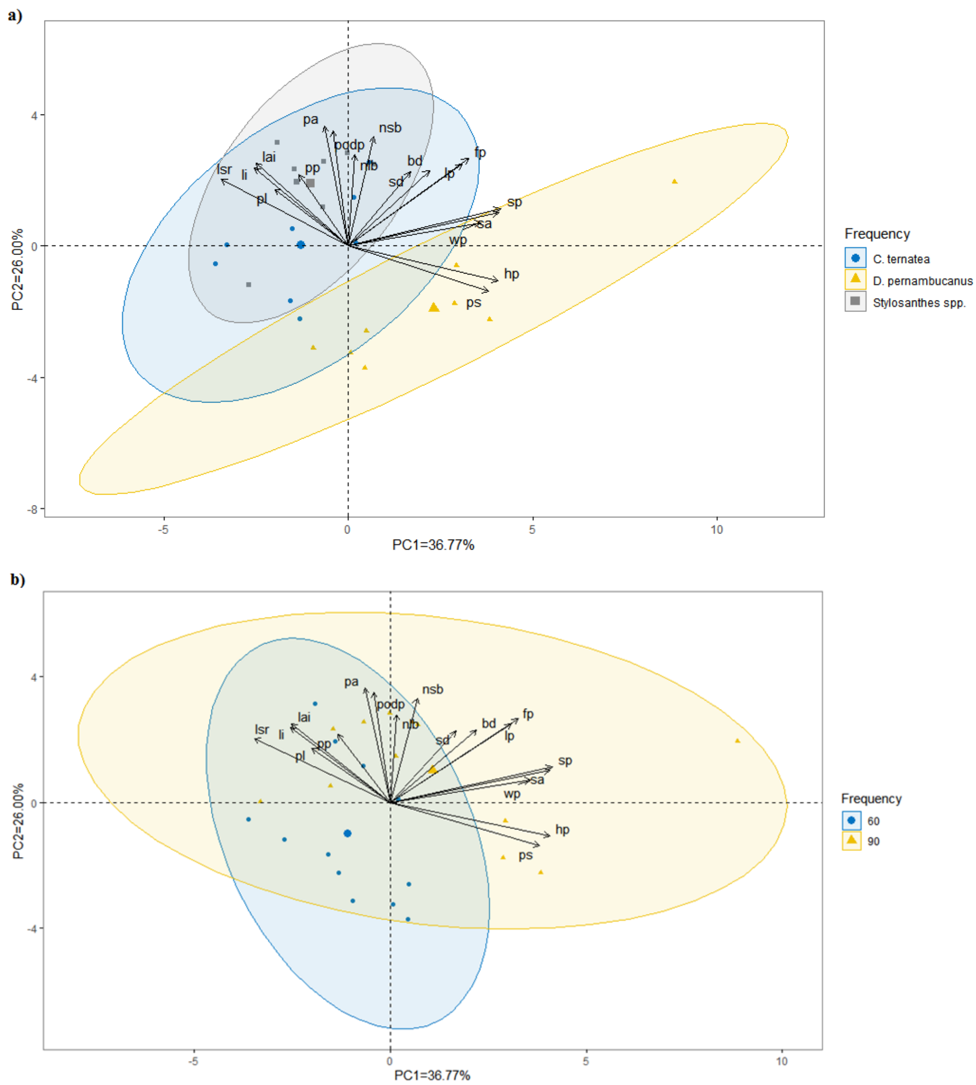

Figure 3 - Principal component analysis based on morphological and productive characteristics of different forage legumes species, managed under two harvesting frequencies (60 and 90 days). Observations: species (A); harvesting frequency (B). Height of the plant (hp), width of the plant (wp), leaf area index (lai), light interception (li), forage production (fp), leaf: stem ratio (lsr), leaf production (lp), stems production (sp), pod production (pp), proportions of leaves, (pl), proportions of stems, (ps) proportions of pods (pp), stems accumulation (sa), pods accumulation (pa), number of secondary branches (nsb), number of leaves per branch (nlb), branch diameter (bd), stem diameter (sd).

sustainability of the pastures composed of legumes, as it helps to form seed banks in the soil (SILVA et al., 2010; TEXEIRA et al., 2010).

CONCLUSION

It was verified in this study that different types of forage legumes, despite their differences in terms of plant structure, and regardless of the harvesting frequency (60 or 90 days), did not show significant differences in terms of their total forage mass accumulation. However, longer harvesting frequencies (90 days) affected differently these legumes, especially the accumulation of stems in *D. pernambucanus*. In general, 90 days harvesting frequency increased the total production of the legumes. The choice of the best harvesting frequency for these legumes evaluated should consider the proposal of the usage. In our study, it was shown that harvesting at 60 days did not affect the total forage mass accumulated, which possibly is the most advantageous choice if the aim is to produce forage with better quality. For seed production, these legumes might be harvested later than 60 days.

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DECLARATION OF CONFLICT OF INTEREST

All authors contributed equally to the manuscript.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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