

# PLANTING DENSITIES AND THE USE OF LOCAL WEEDS AS AN ALTERNATIVE TO ACCELERATE THE INCORPORATION OF GREEN MANURES †

# [DENSIDADES DE SIEMBRA Y APROVECHAMIENTO DE ARVENSES LOCALES COMO ALTERNATIVA PARA ACELERAR LA INCORPORACIÓN DE ABONOS VERDES]

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## SUMMARY

**Background.** Tropical legumes used as green manure require between 2.5 and 4.5 months of growth to be incorporated into the soil, an increase in planting density and the use of weed biomass is expected to help reduce the time required for the incorporation of legumes. Objective. To determine plant survival and dry matter production of five tropical legumes produced in conjunction with local weeds, as a function of planting density and legume age, to identify the most appropriate alternative to accelerate the incorporation of plant biomass in tropical regions. Methodology. The legumes Canavalia ensiformis, Crotalaria juncea, Cajanus cajan, Sesbania sp. and Mucuna aterrima were established in Veracruz, Mexico. Each species was established at five planting densities and biomass was collected at four ages, 30, 60, 90 and 120 days after sowing (DAS). Results. Plant height, leaf/stem ratio, percentage of broadleaf and narrowleaf weeds, as well as dry matter produced by legumes, weeds, and the total of both, changed as a function of plant age. **Implications.** To use green manures in a crop rotation system under rainfed conditions, it is necessary to know the amount of biomass of local weeds and legumes produced over time. The results of the study will help to define the most appropriate cutting age for each of the green manure species evaluated. Conclusion. It is concluded that, independent of planting density, as the age of the legumes increases, plant survival and the leaf/stem ratio decrease, but the amount of dry matter increases. C. cajan and C. juncea have a greater capacity to compete with local weeds, which is attributed to the height they can reach in the first 30 DAS. Regardless of the species and planting density, the maximum production of total dry matter per hectare is achieved between 30 and 60 DAS, when it is recommended to incorporate the biomass to produce under rainfed conditions.

Key words: Cajanus cajan; Crotalaria juncea; C/N ratio; Nitrogen; Organic matter.

## RESUMEN

Antecedentes. Las leguminosas tropicales utilizadas como abono verde requieren entre 2.5 y 4.5 meses de crecimiento para incorporarlas al suelo, se espera que un aumento de la densidad de siembra y el uso de la biomasa de arvenses ayuden a reducir el tiempo requerido para la incorporación de las leguminosas. **Objetivo.** Determinar la supervivencia de plantas y la producción de materia seca de cinco leguminosas tropicales producidas en conjunto con arvenses locales, en función de la densidad de siembra y la edad de la leguminosa, para identificar la alternativa adecuada que permita acelerar la incorporación de la biomasa vegetal en regiones tropicales. **Metodología.** Las leguminosas

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*Canavalia ensiformis, Crotalaria juncea, Cajanus cajan, Sesbania* sp. y *Mucuna aterrima* se establecieron en Veracruz, México. Cada especie se estableció a cinco densidades de siembra y se colectó la biomasa en cuatro edades, 30, 60, 90 y 120 días después de la siembra (DDS). **Resultados.** La altura de la planta, la relación hoja/tallo, el porcentaje de arvenses de hoja ancha y de hoja angosta, así como la materia seca producida por las leguminosas, las arvenses y el total de ambas, cambiaron en función de la edad de la planta. **Implicaciones.** Para utilizar abonos verdes en un sistema de rotación de cultivos, en condiciones de temporal, es necesario conocer la cantidad de biomasa de arvenses y leguminosas producidas a través del tiempo. Los resultados del estudio ayudarán a definir la edad de corte más adecuada para cada una de las especies de abonos verdes evaluadas. **Conclusiones.** Se concluye que, independientemente de la densidad de plantación, a medida que aumenta la edad de las leguminosas, disminuye la supervivencia de las plantas y la relación hoja/tallo, pero aumenta la cantidad de materia seca. *C. cajan y C. juncea* tienen una mayor capacidad para competir con las arvenses locales, lo que se atribuye a la altura que pueden alcanzar en los primeros 30 DDS. Independientemente de la especie y de la densidad de plantación, la máxima producción de materia seca total por hectárea se alcanza entre los 30 y 60 DDS, momento en que se recomienda incorporar la biomasa vegetal para producir en condiciones de temporal.

Palabras clave: Cajanus cajan; Crotalaria juncea; relación C/N; Nitrógeno; Materia orgánica.

# **INTRODUCTION**

Different plant species of the Fabacea family, also known as legumes, can be used for weeding control or as a source of nitrogen (N) for agricultural crops. Legumes form symbioses with atmospheric nitrogenfixing bacteria, this symbiosis allows the use of atmospheric N in the agriculture (Stein et al., 2023). When legumes are used with the objective of protecting the soil they are called "cover crops" and when the objective is to supply N they are called "green manures" (Matías-Ramos et al., 2023). In Mexico, the green manure technology is little used, however, this situation is expected to change in the short term. This change is attributed to the fact that the Government of Mexico since 2019 has implemented different strategies at the national level to change to an agroecological production system. Among these strategies are the "Producción para el bienestar" and "Sembrando vida" programs. Another factor that favors the use of legumes in Mexico are two decrees, issued in 2020 and 2023 (DOF31/12/2020 and DOF13/02/2023), that establish the gradual substitution of the use of the herbicide Glyphosate (Escalona Aguilar et al., 2021; Bartra, 2022; CONAHCYT, 2023). To achieve the substitution of this herbicide, different research has been developed that includes the use of legumes for weed control (CONAHCYT, 2021). In addition to the agricultural use of legumes, they have medicinal uses and serve as human and animal food (Vasconcelos et al., 2020), which increases their benefits to society.

According to Matías-Ramos *et al.* (2023), worldwide, of the research focused on the use of tropical legumes in agriculture, most have focused on 14 species; they also indicate that there are 24 other promising species that can improve the physical, chemical, and biological properties of the soil. The same authors mention that further research is needed to generate new knowledge to take advantage of the multiple benefits of tropical legumes. A study conducted in the central region of

Veracruz, Mexico, where 10 species of tropical legumes were evaluated in monoculture, with weed, pest, and disease control, revealed that, in a period of 75 to 132 days after sowing (DAS) of legumes, between 4 and 16 t ha<sup>-1</sup> of dry matter (DM) can be obtained. It also revealed that, with that amount of DM the N that can be supplied to the soil fluctuates between 73 and 435 kg ha<sup>-1</sup> (Ávila-Escobedo *et al.*, 2022).

It has been reported that the maximum N concentration in legume biomass occurs before the plants reach the full flowering stage (Kaneko et al., 2023). Considering the above, if the objective is to use tropical legumes as a nitrogen source, it is necessary to wait between 2.5 and 4.5 months to cut and incorporate aerial biomass. This time can be inconvenient if it is desired to produce under rainfed conditions, since 70% of Mexico's agricultural area is produced in this modality (SIAP, 2023) and, in tropical areas the rainy period lasts around four to eight months (Delgado-Carranza et al., 2017; Murray-Tortarolo, 2021; Takano-Rojas et al., 2023). This situation restricts the use of legumes only for agricultural crops that have a production cycle between three and five months. In addition to the time required for the incorporation of aerial biomass, other criteria should be considered to select the most suitable legume species. Among these criteria are their ability to compete with local weeds and their susceptibility to attack by pests and diseases (Chapagain et al., 2020; Das et al., 2020). Considering the above, it is necessary to look for strategies to increase the production of plant biomass in the shortest possible time, to expand its use in a greater number of agricultural crops. One strategy that can be employed is to increase planting density (Baath et al., 2023) or to take advantage of the biomass produced by local weeds. Based on the above, the objective was to determine plant survival and DM production of five tropical legumes produced in conjunction with local weeds, as a function of planting density and age of the legumes, to identify the most appropriate alternative to accelerate the incorporation of plant biomass in tropical regions. The hypothesis of

the study is that DM production will increase as planting density increases, which will reduce the time required for the incorporation of plant biomass.

## MATERIALS AND METHODS

The experiment was carried out in the "Campo Experimental Cotaxtla" of the "Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias" in Veracruz, Mexico (18° 56' 13" N 96° 11' 38" W). Precipitation and temperature were recorded during the development of the research (Figure 1). Before planting legumes, the soil had the following characteristics: pH of 5.62; organic matter of 2.88%; loam texture, with 11, 41 and 48% of clay, sand, and silt, respectively. The levels of nitric nitrogen (Brusina), P-PO<sub>4</sub><sup>-</sup> (Bray) and S-SO<sub>4</sub><sup>-2</sup> (Turbidimetric),  $K^{\scriptscriptstyle +}$  (Ac.NH<sub>4</sub> pH 7.0),  $Ca^{\scriptscriptstyle +2}$  (Ac.NH<sub>4</sub> pH 7.0) and  $Mg^{\scriptscriptstyle +2}$ (Ac.NH<sub>4</sub> pH 7.0) were 106, 60, 20, 680, 1,990 and 510 mg kg<sup>-1</sup>, respectively (Peralta Antonio et al., 2023). The soil was not cultivated in the previous two years, it was maintained with local weeds.

Four erect legume species (Canavalia ensiformis (L.) DC., Crotalaria juncea L., Cajanus cajan (L.) Huth and Sesbania sp.) and one creeping species (Mucuna aterrima (Piper and Tracy) Holland) were used. The green manures used were collected since 2019, from seed stores, collections in the central coastal region of Veracruz and from other experimental centers of INIFAP. The species were used in previous experiments. Seeds are refreshed every year. Seeds harvested in autumn - winter 2021 were used for this research. Prior to sowing the legumes, two passes of harrowing were carried out. The distance between furrows was 0.8 m, resulting in a total of 125 furrows per hectare. The legume seeds used in the experiment were obtained from a harvest of the previous cycle, harvested during the first semester of 2022. Prior to sowing, the germination percentage of each legume was determined. All legumes were sown on the same date, July 14, 2022.

The treatments consisted of the interaction of three study factors: legume species (five species), planting density (five densities) and legume cutting age (four cutting ages, carried out at 30, 60, 90 and 120 days after sowing). A randomized block design was used. The treatments were arranged in subdivided plots. The large, medium, and small plots corresponded to species, density, and plant age, respectively. Each treatment had three replicates. The experimental unit consisted of four furrows of 1 linear meter, which corresponds to an area of 3.2 m<sup>2</sup>. The number of seeds deposited per experimental unit varied according to the legume species, fluctuating between 4 and 1248 seeds. For the measurement of variables, the plants present in the two central furrows, which had complete competition, were used.

Seed size was the parameter considered to define the planting density for each species, the individual seed weights for C. ensiformis, M. aterrima, C. cajan, C. juncea, and Sesbania sp. were 1.57, 1.07, 0.10, 0.03 and 0.008 g, respectively. For large-seeded species (C. ensiformis and M. aterrima), density consisted of the number of seeds per linear meter. For small-seeded species, density consisted of seed grams per linear meter (Table 1). With the germination percentage data, for each species, the amount of seed was adjusted to obtain 100% germination at the time of sowing. Postsowing, the water supply depended exclusively on the presence of rainfall, during the experiment a total of 784 mm (122, 465, 84 and 113 mm were precipitated during the first, second, third and fourth month after sowing the legumes). No weed, pest or disease control was performed.

The variables of responses were the number of plants per linear meter, plant height, the presence of local weeds, legume leaf/stem ratio, legume dry matter, local weeds dry matter, and total dry matter. For the recording of the variables, destructive sampling was carried out at four cutting ages, which corresponded to 30, 60, 90, and 120 days after sowing (DAS). In the case of Sesbania sp., only the first three samples were taken, since it completed its productive cycle during that time. To estimate vegetal biomass production, for each sampling date, in each experimental unit, the total number of plants and the fresh weight of the biomass of the legumes in two furrows that had complete competition were quantified. The number of legume plants per linear meter changed as a function of planting density and plant age, from 1 to 200 plants. In the specific case of weeds, the fresh weight of the biomass present in 2 m<sup>2</sup> was recorded. For each sampling date, for leguminous plants and weeds, only living plant tissue that presented a green color or beginning the senescence process (green-yellow color) was considered. To determine the plant height of erect legume species, the height of five representative plants was recorded in each experimental unit. In the case of the creeping species, the height was recorded in five points of the experimental area. Plant height was recorded considering the distance between the soil surface and the highest part of the plant. In each experimental unit, legumes were collected first. Once the legumes were removed, the local weeds were visually classified into broad-leaved and narrowleaved plants. This classification was carried out in each m<sup>2</sup>, taking as a reference the proportion of the soil occupied by broad-leaved or narrow-leaved weeds.

For each sampling date and in each experimental unit, representative samples of legumes and weeds were taken to determine the DM. In the case of erect legume species, the same plants used to measure height, their leaves and stems were separated. In the case of the creeping species, a representative sample of 1 kg was taken, and the leaves and stems were separated. As for weeds, a representative sample of 1 kg was taken. All plant samples were dried in a forced air oven at 70 °C, until reaching constant weight. With the DM of each organ the leaf/stem ratio of the legume was determined, in addition, the legumes DM, local weeds DM and total DM produced per hectare was estimated.

To know the behavior of the different legumes over time a regression analysis (p < 0.05) was performed for each legume species. In all cases, the models with the highest coefficient of determination ( $\mathbb{R}^2$ ) were selected. Regression analysis was performed with Excel and graphs with GraphPad Prism 8.

### RESULTS

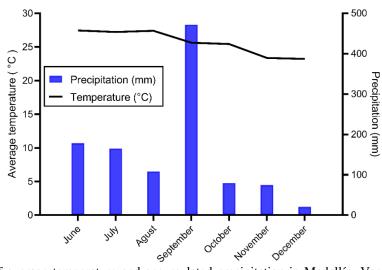
During the experimental period, the average initial temperature was 28°C, during July, at the end of the experiment it decreased to 23°C, in November (Figure 1). Total precipitation during the experiment was around 760 mm. The amount of rainfall during 30, 60, 90 and 120 DAP were 432, 219, 66 and 42 mm, respectively (Figure 1).

## Number of plants per linear meter

In the case of *C. cajan, C. ensiformis* and *M. aterrima*, independent of sampling date, the number of plants increased as planting density increased (Figure 2 a, b, d). In all three cases, linear models explained the behavior of the number of plants per linear meter, with the  $R^2$  between 0.79 and 0.98.

Table 1. Number or weight of seeds of five tropical legume species deposited per linear meter at the time of sowing, in Veracruz, Mexico.

Especie	Density 1	Density 2	Density 3	Density 4	Density 5
	Number of seeds per linear meter				
C. ensiformis	2.0	4.0	5.0	8.0	10.0
M. aterrima	1.0	3.0	5.0	7.0	10.0
	Grams of seeds per linear meter				
C. juncea	1.0	2.0	3.0	4.0	5.0
C. cajan	2.0	4.0	6.0	8.0	10.0
Sesbania sp.	0.5	1.0	1.5	2.0	2.5
	Equivalent to number of seeds per linear meter				
C. juncea	33	66	99	132	166
C. cajan	20	40	60	80	100
Sesbania sp.	63	126	190	252	312



**Figure 1.** Behavior of average temperature and accumulated precipitation in Medellín, Veracruz, Mexico between June and December 2022.

For C. juncea and Sesbania sp. only in the first sampling, no greater number of plants per m<sup>2</sup> was obtained with the highest planting density (Figure 2 c, e). A third-degree polynomial model was the main explanation for the behavior of the number of plants per linear meter for these two species. The R<sup>2</sup> remained between 0.88 and 0.97.

30DAS y = 15.106x - 12.394 R<sup>2</sup> = 0.9079

0-

60

50

(C)

Plants per meter

10 0-

0

0

2

Crotalaria juncea

33

66

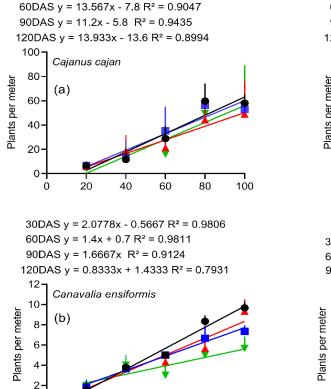
99

Seeds deposited per linear meter

132

165

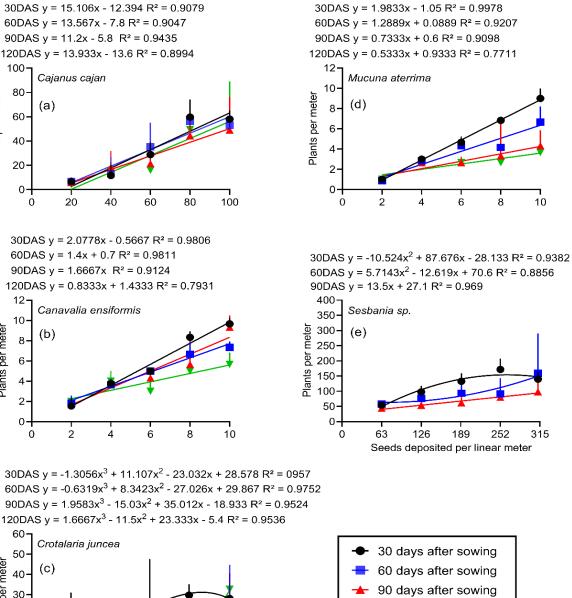
Δ



6

8

Regardless of the species and planting density, the highest number of plants per m<sup>2</sup> was detected in the first sampling carried out, that is, at 30 DAS (Figure 2 a, b, c, d, e).

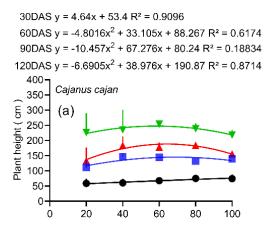


120 days after sowing

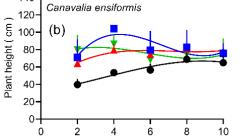
Figure 2. Number of plants per m<sup>2</sup> of five tropical legume species, planted at different planting densities and sampled at different days after sowing (DAS).

### **Plant hight**

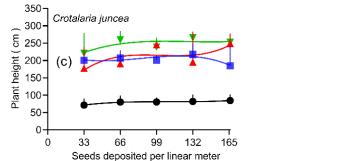
In all legume species, plant height depended mainly on age, in all cases, the lowest height was detected at 30 DAS while the greatest height was reached after 60 DAS. *C. cajan* and *C. juncea* were the species that achieved the greatest plant height, while the lowest



30DAS y = -0.4667x<sup>3</sup> + 2.3286x<sup>2</sup> + 6.7952x + 31.667 R<sup>2</sup> = 0.9413 60DAS y =  $3.994x^3 - 39.693x^2 + 115.51x - 6.9956$  R<sup>2</sup> = 0.6682 90DAS y =  $1.0231x^3 - 10.95x^2 + 37.526x + 37.311$  R<sup>2</sup> = 0.7873 120DAS y =  $1.3662x^3 - 11.749x^2 + 26.496x + 64.658$  R<sup>2</sup> = 0.482 140 T

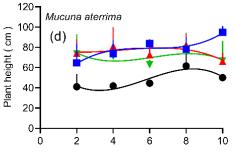


 $\begin{array}{l} 30 \text{DAS y} = 0.7722 x^3 - 7.7119 x^2 + 25.583 x + 52.893 \ \text{R}^2 = 0.9864 \\ 60 \text{DAS y} = -3.075 x^3 + 23.642 x^2 - 50.55 x + 231.93 \ \text{R}^2 = 0.7365 \\ 90 \text{DAS y} = 5.1991 x^3 - 48.446 x^2 + 147.44 x + 68.356 \ \text{R}^2 = 0.5934 \\ 120 \text{DAS y} = 1.6278 x^3 - 18.74 x^2 + 69.932 x + 169.93 \ \text{R}^2 = 0.5886 \end{array}$ 

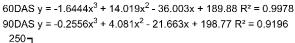


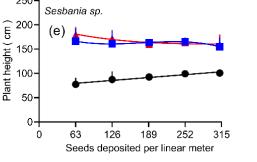
plant height was detected in *M. aterrima*. For most of the legume species, the behavior of plant height was mainly fitted to a third-degree polynomial model. Most models presented  $R^2$  between 0.58 and 0.99, however, in the case of *C. cajan* and *M. aterrima*, for sampling at 90 and 120 DAS the  $R^2$  was lower than 0.50 (Figure 3 a, b, c, d, e).

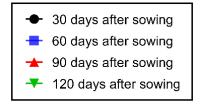
 $\begin{array}{l} 30 \text{DAS y} = -2.5222 x^3 + 21.962 x^2 - 51.316 x + 73.667 \ \text{R}^2 = 0.847 \\ 60 \text{DAS y} = 1.8278 x^3 - 16.46 x^2 + 49.646 x + 28.88 \ \text{R}^2 = 0.9175 \\ 90 \text{DAS y} = -0.8333 x^3 + 5.5437 x^2 - 9.4008 x + 79.967 \ \text{R}^2 = 0.4973 \\ 120 \text{DAS y} = -1.7731 x^3 + 16.333 x^2 - 44.727 x + 104.77 \ \text{R}^2 = 0.354 \end{array}$ 



30DAS y = 5.8667x + 73.827 R<sup>2</sup> = 0.9465







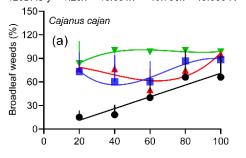
**Figure 3.** Plant height of five tropical legume species planted at different planting densities and sampled at different days after sowing (DAS).

#### Local weeds

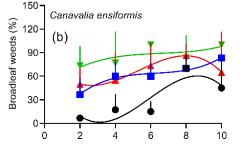
Regarding local weeds, in all areas where legumes were established, independent of the species, the lowest proportion of broadleaf weeds was present at 30 DAS, while the highest proportion was present at 120 DAS (Figure 4 a, b, c, e). As for narrowleaf weeds, in the areas where *C. cajan*, *C. ensiformis*, *C. juncea*, and *Sesbania* sp. were established, the highest proportion was detected at 30 DAS, mainly at the lowest planting density (Figure 5 a, b, c, e). The only exception was *M*.

30DAS y = 14.967x - 3.8333 R<sup>2</sup> = 0.9178

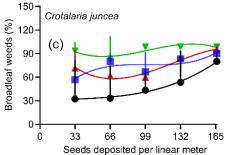
60DAS y = -3.1944x<sup>3</sup> + 32.798x<sup>2</sup> - 94.008x + 138.67 R<sup>2</sup> = 0.9203 90DAS y = 1.8056x<sup>3</sup> - 9.7024x<sup>2</sup> + 6.8254x + 79.667 R<sup>2</sup> = 0.7472 120DAS y = 1.25x<sup>3</sup> - 13.631x<sup>2</sup> + 46.786x + 49.333 R<sup>2</sup> = 0.9436



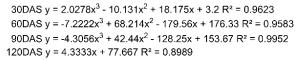
 $\begin{array}{l} 30 \text{DAS y} = -5.5833x^3 + 49.25x^2 - 112.83x + 78.8 \ \text{R}^2 = 0.7761 \\ 60 \text{DAS y} = 2.2222x^3 - 20.714x^2 + 67.063x - 11.333 \ \text{R}^2 = 0.9804 \\ 90 \text{DAS y} = -4.0278x^3 + 32.083x^2 - 63.889x + 86 \ \text{R}^2 = 0.9977 \\ 120 \text{DAS y} = 1.1111x^3 - 10.952x^2 + 37.937x + 43.333. \ \text{R}^2 = 0.6059 \end{array}$ 

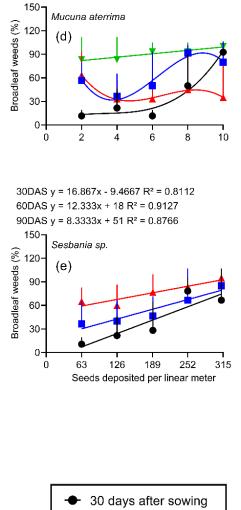


 $\begin{array}{l} 30 \text{DAS y} = 0.6389 x^3 - 2.0833 x^2 + 4.6111 x + 28.8 \ \text{R}^2 = 0.9939 \\ 60 \text{DAS y} = 2.2222 x^3 - 20.238 x^2 + 60.873 x + 15.333 \ \text{R}^2 = 0.7756 \\ 90 \text{DAS y} = -1.9444 x^3 + 22.262 x^2 - 67.46 x + 119.67 \ \text{R}^2 = 0.9437 \\ 120 \text{DAS y} = -2.0833 x^3 + 19.107 x^2 - 48.81 x + 124.33 \ \text{R}^2 = 0.7458 \end{array}$ 



*aterrima*, specifically in the sampling conducted at 90 DAS, where the percentage of weeds showed little variation among the different planting densities (Figure 5 d). For most of the legume species, the behavior of the presence of broadleaf and narrowleaf weeds over time was mainly adjusted to a third-degree polynomial model. The exception was Sesbania sp. which fitted a linear model. Most of the models the  $R^2$  remained between 0.60 and 0.99 (Figure 4 and 5 a, b, c, d, e).





🛨 90 days after sowing

60 days after sowing

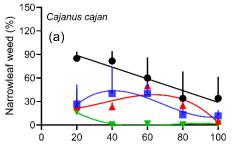
➡ 120 days after sowing

**Figure 4.** Proportion of broadleaf weeds present at the time of cutting of aerial biomass of five tropical legume species, sown at different planting densities and sampled at different days after sowing (DAS).

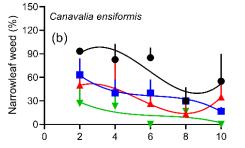
#### Legume leaf/stem ratio

A greater variation in leaf/stem ratio values was detected with *C. cajan*, *C. ensiformis*, *C. juncea* and *Sesbania sp*. In the first three species, the highest values were detected at 30 DAS, while the lowest leaf/stem ratio occurred at 120 DAS (Figure 6 a, b, c). In the case of *Sesbania* sp. there was little variation in the values detected at 30 and 60 DAS, however, both were higher than the values detected at 90 DAS (Figure

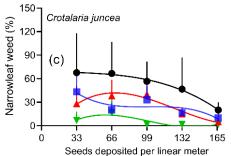
 $\begin{array}{l} 30 \text{DAS y} = -14.967 x + 103.83 \ \text{R}^2 = 0.9178 \\ 60 \text{DAS y} = 3.1944 x^3 - 32.798 x^2 + 94.008 x - 38.667 \ \text{R}^2 = 0.9203 \\ 90 \text{DAS y} = -1.8056 x^3 + 9.7024 x^2 - 6.8254 x + 20.333 \ \text{R}^2 = 0.7472 \\ 120 \text{DAS y} = -1.25 x^3 + 13.631 x^2 - 46.786 x + 50.667 \ \text{R}^2 = 0.9436 \end{array}$ 

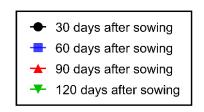


 $\begin{array}{l} 30\text{DAS } y = 5.5833x^3 - 49.25x^2 + 112.83x + 21.2 \ \text{R}^2 = 0.7761 \\ 60\text{DAS } y = -2.2222x^3 + 20.714x^2 - 67.063x + 111.33 \ \text{R}^2 = 0.9804 \\ 90\text{DAS } y = 4.0278x^3 - 32.083x^2 + 63.889x + 14 \ \text{R}^2 = 0.9977 \\ 120\text{DAS } y = -1.1111x^3 + 10.952x^2 - 37.937x + 56.667 \ \text{R}^2 = 0.6059 \end{array}$ 



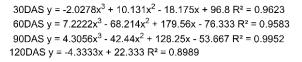
 $\begin{array}{l} 30\text{DAS y} = -0.6389x^3 + 2.0833x^2 - 4.6111x + 71.2 \ \text{R}^2 = 0.9939 \\ 60\text{DAS y} = -2.2222x^3 + 20.238x^2 - 60.873x + 84.667 \ \text{R}^2 = 0.7756 \\ 90\text{DAS y} = 1.9444x^3 - 22.262x^2 + 67.46x - 19.667 \ \text{R}^2 = 0.9437 \\ 120\text{DAS y} = 2.0833x^3 - 19.107x^2 + 48.81x - 24.333 \ \text{R}^2 = 0.7458 \end{array}$ 

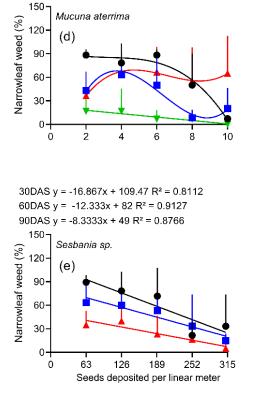




**Figure 5.** Proportion of narrowleaf weeds present at the time of cutting of aerial biomass of five tropical legume species, sown at different planting densities and sampled at different days after sowing (DAS).

6 e). The least variation in leaf/stem ratio was observed with *M. aterrima*, only a small variation was detected at the second planting density, where the highest values were detected at 30 and 60 DAS (Figure 6 d). For most legume species, the behavior of the plant leaf/stem ratio over time was mainly fitted to a thirddegree polynomial model. Most models the R<sup>2</sup> ranging from 0.60 to 0.99. The only exception was *M. aterrima*, sampled at 30 and 60 DAS, where the R<sup>2</sup> were 0.25 and 0.27, respectively (Figure 6 a, b, c, d, e).

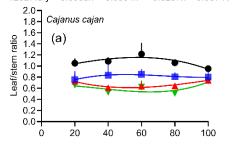




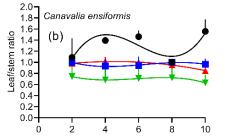
#### Legume dry matter

Regardless of the legume species, the amount of DM increased with increasing plant age. In the case of *C. cajan, C. ensiformis*, and *C. juncea*, the highest amount of DM was detected at 90 or 120 DAS (Figure 7 a, b, c). For *M. aterrima*, the highest DM values were obtained at 120 DAS, although high values were also obtained at 60 and 90 DAS with the highest planting densities (Figure 7 d). For *Sesbania* sp. the highest DM amount was detected at 60 DAS (Figure 7 e). Of the five legume species, the highest DM amount was obtained with *C. cajan* and *C. juncea* (Figure 7 a, c).

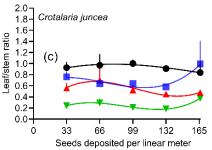
$$\begin{split} &30\text{DAS y} = -0.0039x^3 - 0.0054x^2 + 0.1295x + 0.9207 \ \text{R}^2 = 0.7938 \\ &60\text{DAS y} = 0.0079x^3 - 0.0878x^2 + 0.2916x + 0.5423 \ \text{R}^2 = 0.9729 \\ &90\text{DAS y} = -0.0042x^3 + 0.0666x^2 - 0.2646x + 0.9225 \ \text{R}^2 = 0.9223 \\ &120\text{DAS y} = 0.0095x^3 - 0.0504x^2 + 0.0257x + 0.657 \ \text{R}^2 = 0.6012 \end{split}$$



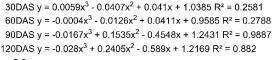
 $\begin{aligned} &30\text{DAS } y = 0.104x^3 - 0.9376x^2 + 2.519x - 0.6222 \ \text{R}^2 = 0.7925 \\ &60\text{DAS } y = -0.0142x^3 + 0.1358x^2 - 0.3818x + 1.2599 \ \text{R}^2 = 0.9339 \\ &90\text{DAS } y = -0.0006x^3 - 0.0163x^2 + 0.0842x + 0.915 \ \text{R}^2 = 0.9983 \\ &120\text{DAS } y = -0.016x^3 + 0.1391x^2 - 0.3668x + 0.9884 \ \text{R}^2 = 0.9999 \end{aligned}$ 

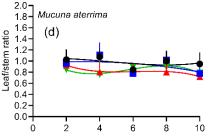


 $\begin{array}{l} 30 \text{DAS y} = \ 0.0025 x^3 - 0.0477 x^2 + 0.1865 x + 0.783 \ \text{R}^2 = 0.9371 \\ 60 \text{DAS y} = \ 0.0292 x^3 - 0.1901 x^2 + 0.294 x + 0.6174 \ \text{R}^2 = 0.9358 \\ 90 \text{DAS y} = 0.0342 x^3 - 0.3169 x^2 + 0.8192 x + 0.0303 \ \text{R}^2 = 0.9341 \\ 120 \text{DAS y} = 0.029 x^3 - 0.2367 x^2 + 0.556 x - 0.1072 \ \text{R}^2 = 0.9998 \end{array}$ 

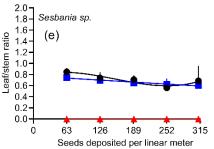


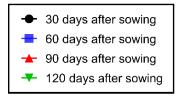
Little effect of planting density on legume biomass production was detected. With *C. cajan* and *C. juncea*, the effect was only observed at 30 DAS, with *M. aterrima* the effect was only detected at 60 and 90 DAS (Figure 7 a, c, d); in all the cases mentioned above, biomass increased as planting density increased. With *Sesbania* sp. no effect of planting density on biomass production was detected (Figure 7 e). For most legume species, the behavior of DM production over time was mainly fitted to a third-degree polynomial model. For most models, the  $\mathbb{R}^2$  ranged between 0.65 and 0.99 (Figure 7 a, b, c, d, e).





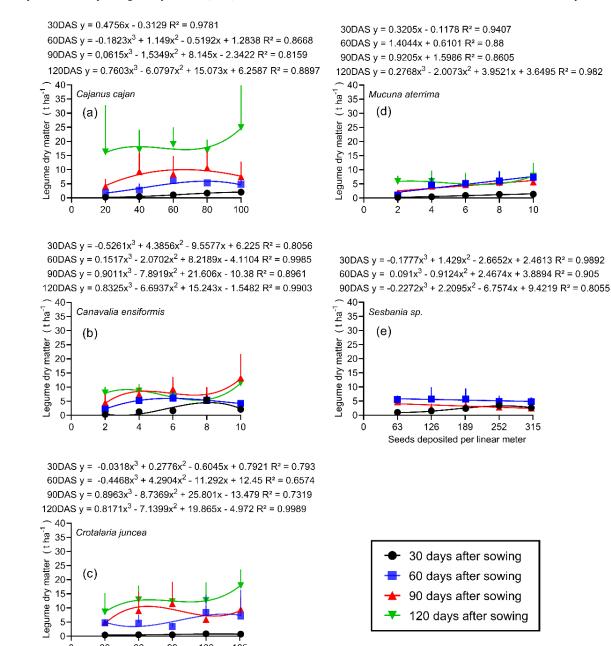
30DAS y =  $0.0159x^3 - 0.1178x^2 + 0.174x + 0.7669 R^2 = 0.8834$ 60DAS y =  $-0.0354x + 0.7705 R^2 = 0.9552$ 





**Figure 6.** Leaf/stem ratio of five tropical legume species, planted at different planting densities and sampled at different days after sowing (DAS).

Pedraza-Monroy et al., 2024



**Figure 7.** Dry matter of five tropical legume species, sown at different planting densities and cut at different days after sowing (DAS).

### Local weeds dry matter

0

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66

99

Seeds deposited per linear meter

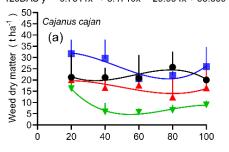
Regardless of the legume species and planting density, the highest amount of DM of weeds was obtained in the first 60 DAS, after this date the values decreased (Figure 8 a, b, c, d, e).

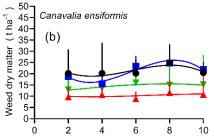
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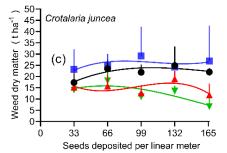
The effect of planting density on weed biomass production was not consistent, it varied according to the species and sampling date. The only ones that showed similar trends were *C. cajan* and *M. aterrima*, in three of the four samplings conducted, higher weed biomass was detected at the lowest planting density (Figure 8 a, d). In the case of *C. ensiformis, C. juncea*, and *Sesbania* sp. for the different sampling dates, there was little variation in the amount of weed biomass with the highest and lowest legume planting density (Figure 8 b, c, e). For most legume species, the behavior of weed DM production over time was mainly fitted to a third-degree polynomial model. For most models, the  $R^2$  ranged between 0.63 and 0.98, although, the exceptions were *C. ensiformis* sampled at 90 DAS and *C. juncea* sampled at 60 and 90 DAS, where the  $R^2$  were 0.23, 0.47 and 0.44, respectively (Figure 8 a, b, c, d, e).

 $\begin{aligned} &30\text{DAS y} = -0.8647x^3 + 7.3532x^2 - 17.615x + 32.653 \ \text{R}^2 = 0.6738 \\ &60\text{DAS y} = 0.7826x^3 - 5.4557x^2 + 7.0284x + 29.677 \ \text{R}^2 = 0.9005 \\ &90\text{DAS y} = 0.3958x^3 - 2.9411x^2 + 4.4964x + 17.743 \ \text{R}^2 = 0.6555 \\ &120\text{DAS y} = -0.7311x^3 + 8.4719x^2 - 29.994x + 38.309 \ \text{R}^2 = 0.9815 \end{aligned}$ 





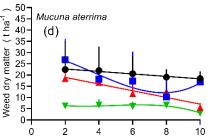
30DAS y =  $0.1497x^3 - 2.3073x^2 + 10.366x + 9.464 R^2 = 0.7688$ 60DAS y =  $0.4333x^3 - 4.4012x^2 + 13.899x + 12.873 R^2 = 0.472$ 90DAS y =  $-0.8292x^3 + 7.0244x^2 - 17.313x + 26.903 R^2 = 0.442$ 120DAS y =  $0.1472x^3 - 2.1988x^2 + 6.7706x + 9.9167 R^2 = 0.6833$ 



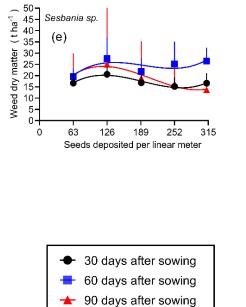
30DAS y = -1.096x + 23.792 R<sup>2</sup> = 0.9664 60DAS y = 0.5028x<sup>3</sup> - 2.769x<sup>2</sup> - 1.4385x + 30.05 R<sup>2</sup> = 0.881

90DAS y = -2,979x + 21.953 R<sup>2</sup> = 0.8871

120DAS y =  $-0.2667x^3 + 1.9195x^2 - 4.0638x + 8.8067 R^2 = 0.906$ 



30DAS y =  $0.8992x^3 - 8.2977x^2 + 21.906x + 2.2967 R^2 = 0.9334$ 60DAS y =  $0.9667x^3 - 9.0214x^2 + 25.895x + 2.1933 R^2 = 0.6325$ 90DAS y =  $0.9292x^3 - 9.1744x^2 + 24.646x + 3.98 R^2 = 0.9328$ 



120 days after sowing

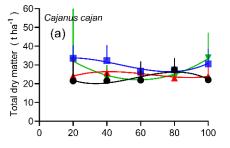
**Figure 8.** Dry matter of local weed presents in the same site where five tropical legume species were grown, sown at different planting densities, and cut at different days after sowing (DAS).

## Total dry matter

Considering together the DM of legumes and local weeds, the effect of planting density of legumes was minimal. Only with *C. juncea*, at 60 DAS, an increase in biomass production was detected with increasing plant density. Differences in DM production was detected mainly by the effect of plant age. With *C. ensiformis*, *C. juncea*, *M. aterrima* and *Sesbania* sp. the highest amounts were obtained in the first 60 DAS (Figure 9 b, c, d, e).

A slight difference was observed with *C. cajan*, as high DM values were also obtained at 120 DAS, specifically with the highest planting density (Figure 9 a). For most legume species, the behavior of total plant dry matter production over time was mainly fitted to a third-degree polynomial model. For most models, the  $R^2$  ranged between 0.65 and 0.98. The exceptions were *C. juncea* and *M. aterrima* in the sampling conducted at 120 DAS, where the  $R^2$  were 0.44 and 0.40, respectively (Figure 9 a, b, c, d, e).

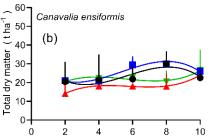
30DAS y =  $-0.9185x^3 + 7.8626x^2 - 18.56x + 33.419$  R<sup>2</sup> = 0.7432 $60DAS y = 0.6003x^3 - 4.3066x^2 + 6.5091x + 30.96 R^2 = 0.9211$ 90DAS y =  $0.4573x^3 - 4.476x^2 + 12.641x + 15.401 R^2 = 0.8201$ 120DAS y =  $0.0292x^3 + 2.3922x^2 - 14.92x + 44.567 R^2 = 0.8858$ 



30DAS y =  $-1.273x^3 + 10.812x^2 - 24.891x + 36.322 R^2 = 0.7604$  $60DAS y = -1.1316x^3 + 9.0631x^2 - 18.031x + 30.81 R^2 = 0.9243$ 90DAS y =  $0.84x^3 - 7.2681x^2 + 20.024x + 0.5931 R^2 = 0.9983$ 120DAS y =  $0.7867x^3 - 6.6146x^2 + 16.751x + 9.6118 R^2 = 0.8997$ 

 $30DAS y = 0.1179x^3 - 2.0297x^2 + 9.7618x + 10.256 R^2 = 0.7632$ 

90DAS y =  $0.0671x^3 - 1.7125x^2 + 8.4876x + 13.425 R^2 = 0.9043$ 120DAS y =  $0.9643x^3 - 9.3387x^2 + 26.636x + 4.9447 R^2 = 0.4407$ 



60DAS y = 1.5317x + 26.723 R<sup>2</sup> = 0.9293

Crotalaria juncea

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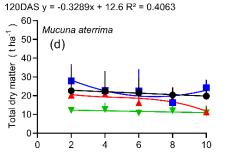
10 0

(tha<sup>1</sup> 50

dry matter

Total

30DAS y = -0.7755x + 23.674 R<sup>2</sup> = 0.9628  $60DAS y = 0.7961x^3 - 5.7145x^2 + 8.7216x + 23.594 R^2 = 0.8064$  $90DAS y = -0.6088x^2 + 1.5943x + 19.29 R^2 = 0.804$ 



30DAS y = 0.7215x<sup>3</sup> - 6.8687x<sup>2</sup> + 19.241x + 4.7579 R<sup>2</sup> = 0.8612  $60DAS y = 1.0577x^3 - 9.9338x^2 + 28.363x + 6.0827 R^2 = 0.6558$ 90DAS y =  $-0.6469x^2 + 1.3218x + 25.196 R^2 = 0.8876$ 

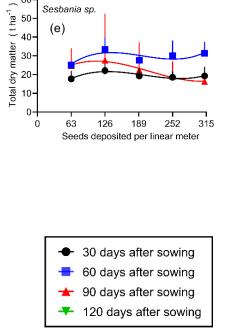


Figure 9. Total dry matter, which includes the dry matter of local weeds and different tropical legumes, sown at different planting densities and cut at different days after sowing (DAS).

60

## DISCUSSION

66

99

Seeds deposited per linear meter

132

165

Edaphoclimatic conditions have different effects on plant development; therefore, plant species established in environments different from their own can be benefited or affected depending on the biotic or abiotic factors presented during their development (de Melo et al., 2023). For this study, the number of plants present on each sampling date allows us to understand the response of legumes as they interact with the different biotic and abiotic factors presented in the study region. With the initial amount of seeds deposited at the time of sowing and the number of plants at the time of sampling, the percentage of plant survival can be estimated. In general, the highest survival was achieved with large-seeded species (C. ensiformis and *M. aterrima*) compared to small-seeded species, since the number of plants of C. juncea, C. cajan, Sesbania sp., C. ensiformis and M. aterrima was equivalent to 29, 66, 83, 92 and 97% of the initial number, respectively. According to the results, planting density and plant age influenced their survival. In the case of C. ensiformis, C. juncea, M. aterrima and Sesbania sp. the highest survival was observed with the lowest planting density, which on average represented 92, 29, 97 and 83% of the initial number of plants,

respectively. The only exception to this behavior was C. ensiformis, since its highest and lowest survival was with the fourth and first planting density, representing 66 and 30% of the initial number of plants, respectively. On the other hand, independently of the legume species, a higher plant survival was detected in the first 60 DAS, since the plants of C. juncea, C. cajan, Sesbania sp., C. ensiformis and M. aterrima present at the time of sampling were equivalent to 27, 50, 69, 91 and 96% of the initial plants, respectively. Legume survival can be attributed to different factors, e.g., stress due to excess or deficit water, temperature stress, interplant competition, and susceptibility to pests and diseases (Chapagain et al., 2020; Das et al., 2020). For this study, the main abiotic factor was excess moisture. The slope of the land used is less than 1%, and during the first 60 DAS a total of 588 mm of rainfall occurred, equivalent to 75% of the total water precipitation in the whole experiment, which caused the death of plants by anoxia, mainly in small-seeded legumes (C. cajan, C. juncea, and Sesbania sp.). The biotic factors that influenced plant development were the presence of herbivores and weeds. In the case of C. cajan, in the germination stage it was attacked by rabbits and although the plants did not die, their development was delayed. As for Sesbania sp. and M. aterrima, at different stages of the experiment they were attacked by different herbivorous insects belonging to the order Coleoptera.

All legumes competed with local weeds during the experiment and the competition capacity changed depending on the species, planting density and plant age, which was reflected in DM production. Considering the total DM produced per hectare, in all legume species, the maximum amount of DM was reached in the first 60 DAS, however, no trend in the effect of planting densities was detected. C. cajan and M. aterrima reached maximum DM production at the lowest planting density (32 and 28 t ha<sup>-1</sup>, respectively), for C. juncea it was detected at the highest planting density (34 t ha<sup>-1</sup>), while for C. ensiformis and Sesbania sp. the maximum DM production (30 and 33 t ha<sup>-1</sup>, respectively) was detected at intermediate planting densities. Considering all species and planting densities, although the maximum amounts of DM were obtained in the first 60 DAS, the greatest proportion of DM corresponded to local weeds. At this time, legumes contributed the smallest proportion, the amounts of C. cajan, C. ensiformis, C. juncea, M. aterrima and Sesbania sp. corresponded to 1 - 9, 2 - 9, 1 - 4, 2 - 4 and 1 - 7% of the total amount produced, respectively. To achieve the maximum contribution of DM through the legumes, it had to elapse between 90 and 120 DAS, reaching values of 16 - 25, 5 - 11, 5 -18, 6-8, and 3-5 t ha<sup>-1</sup> with C. cajan, C. ensiformis, C. juncea, M. aterrima and Sesbania sp. that corresponded to 46 - 76, 32 - 48, 33 - 72, 38 - 66 and 19 - 24% of the total DM produced, respectively. This information is important for decision making, especially if the intention is to produce under rainfed conditions. It should be considered that the rainy season in tropical regions of Mexico lasts between four and eight months (Delgado-Carranza et al., 2017; Murray-Tortarolo, 2021; Takano-Rojas et al., 2023). Therefore, if the intention is to use legumes as a source of N, it is necessary to wait at least 90 DAS to ensure a greater production of DM and thus achieve a greater supply of this nutrient (Ávila-Escobedo et al., 2022). According to the results, C. ensiformis (placing 5 seeds per linear meter), C. juncea (placing 2 to 5 g of seed per linear meter) or C. cajan (placing 2 to 10 g of seed per linear meter) can be used so that the DM obtained fluctuates between 11 and 25 t ha<sup>-1</sup>, which corresponds to between 43 and 76% of the total DM produced per hectare. With these amounts of DM, for these same species, in the same region, and using a slightly lower fertility soil (52, 30, and 18% sand, clay, and silt, respectively; pH of 5.46; organic matter of 3.69%; 16.8, 12. 0, 1956, and 413 mg kg<sup>-1</sup> of P, K, Ca, and Mg respectively) compared to that of this research (41, 11, and 48% sand, clay, and silt, respectively; pH of 5.62; organic matter of 2.88%; 60, 680, 1990, and 510 mg kg<sup>-1</sup> of P, K, Ca, and Mg respectively), Ávila-Escobedo et al. (2022) report an N supply that fluctuates between 217 and 308 kg ha<sup>-1</sup>. On the other hand, if the intention is to produce agricultural crops with a phenological cycle longer than three months, the biomass of the legume will have to be cut at 60 DAS. The most suitable species are *M. aterrima* (placing 7 to 10 seeds per linear meter), C. cajan (placing 6 seeds per linear meter) and C. juncea (placing between 4 and 5 g of seed per linear meter), so that the DM obtained fluctuates between 6 and 8 t ha<sup>-1</sup>, which corresponds to between 21 and 45% of the total DM produced per hectare. With these amounts of DM, for these same species, in the same region, Ávila-Escobedo et al. (2022) report an N supply that fluctuates between 72 and 187 kg ha<sup>-1</sup>.

The results of this study demonstrate the importance of local weeds from a nutritional point of view since they can be an alternative to improve the level of soil organic matter. In 1 hectare of land, at a depth of 30 cm and a bulk density of 1 g cm<sup>-3</sup>, there are 3000 t of soil, therefore, if the aim is to increase the concentration of organic matter by 1%, 30 t of organic matter should be applied. In this study, independent of the legume species and planting density used, between the first 30 and 60 DAS the amount of DM supplied to the soil fluctuated between 16 and 34 t ha<sup>-1</sup> and of this amount, between 55 and 99% corresponded to local weeds. This suggests that the interaction of tropical legumes and local weeds is a viable alternative from the economic and operational point of view to increase the level of organic matter in the soil, since the investment would consist of acquiring seeds, soil preparation, sowing, and incorporation of legumes.

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Considering the DM produced by legumes, C. cajan and C. juncea were the species with the greatest capacity to compete with local weeds, which can be attributed to the height that these species reach. At 60 DAS, C. cajan and C. juncea plants had already reached a height of 1.1 - 1.4 and 1.8 - 2.2 m, values that increased to 2.2 - 2.5 and 2.2 - 2.7 m at 120 DAS, respectively. In the first 30 DAS, independent of the legume species, of the weeds present, the highest proportion were grasses, mainly Sorghum halepense and Cyperus sp. species, which are characterized by their highly competitive ability (Shi et al., 2021; Yazlik and ÜremiŞ, 2022). As time elapsed, the proportion of grasses decreased, and the proportion of broadleaf weeds increased. In this study, at 60 DAS, S. halepense reached a height of between 2.3 and 2.8 m, causing competition for light with legumes. The species most affected by this light competition were C. ensiformis and M. aterrima, since the maximum height reached during the entire experiment fluctuated between 0.4 and 1.4 m. In the case of Sesbania sp. the maximum height reached fluctuated between 0.8 and 1.8 m, however, its phenological cycle was short (less than 4 months), therefore, it did not continue to compete with the weeds. If the objective is to control weeds, the above information suggests using C. cajan and C. juncea in areas where weeds that reach heights greater than 2 m predominate, while C. ensiformis and M. aterrima are recommended for areas where weeds that do not exceed 1.4 m in height predominate.

The plant leaf/stem ratio indicates the units of leaf DM produced for each unit of stem DM produced. The leaf/stem ratio is negatively correlated with the C/N ratio of legume biomass; as the leaf/stem ratio increases, the C/N ratio decreases (Ávila-Escobedo et al., 2022). This characteristic is important from a nutritional point of view, since C/N ratio is negatively correlated with N mineralization (Watthier et al., 2022); N mineralization decreases as the C/N ratio of plant biomass increases. Considering that the highest leaf/stem ratio occurs at 30 DAS and decreases through time, it is speculated that, a lower proportion of N will be released to the soil as legume age increases, mainly for C. cajan, C. ensiformis and C. juncea. This information is useful to be able to take advantage of the N supplied through legumes, since there must be synchronized between N release and the crop requirement (Watthier et al., 2023). For example, if the crop of interest is corn, to obtain the highest forage and grain yield, fertilization should be concluded no later than 70 DAS (Aragão et al., 2022), therefore, better synchronization is expected when legume biomass is cut in the first 30 DAS. The situation is different with perennial or semi-perennial crops. For example, if the crop of interest is pineapple, in Mexico it is recommended to conclude fertilization before the flowering stage, between 7 and 14 months after planting (Rebolledo-Martínez et al., 2023). In this case, better synchrony is expected when biomass is cut at 120 DAS.

## CONCLUSION

According to the results, it is concluded that, independent of planting density, as the age of the legumes increases, plant survival and the leaf/stem ratio decrease, but the amount of dry matter increases. *Cajanus cajan* and *Crotalaria juncea* have a greater capacity to compete with local weeds, which is attributed to the height they can reach in the first 30 days after planting. Regardless of the legume species and planting density, when taking advantage of local weeds, the maximum production of total dry matter per hectare is achieved between 30 and 60 days after planting, at which time it is recommended to incorporate the aerial biomass to produce under rainfed conditions.

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