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Article

Growth analysis of bird's-foot trefoil at different sowing densities

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Abstract

The aerial part of *Lotus corniculatus* L. genotype 202700 of erect habit originating in Uruguay was studied, a growth analysis was performed, evaluating the variables DM yield (DMY), height with ruler (HR), height with plate (HP), yields of leaf (L), stem (S), weeds (W), dead material (DM), (L:S) ratio and intercepted radiation (IR). Immediately after the uniformity cut at 5 cm, continuous weekly cuts were made during a regrowth cycle (eight weeks) in spring. This research was carried out in 'Predio Nuevo' of the College of Postgraduates, Montecillo, Texcoco, State of Mexico, from April 12 to May 31, 2021. Data were analyzed with SAS, according to a completely randomized experimental design in split plots at two densities as treatments (D1= 12 000 plants ha^{-1} and D2= 16 000 plants ha^{-1}) and eight repetitions for each treatment. According to the results, the treatment D2 was the one that presented the highest values 5 450 kg DM ha^{-1}, followed by the treatment D1 with 4 315 kg DM ha^{-1}, the intercepted radiation (%) by treatment 77% for D1 and 83% for D2, reaching the peak at 49 days (92%), the height of the plants behaved positively as the meadow developed. It is concluded that the density with the best overall behavior was D2, which showed the greatest response in terms of the variables leaf, stem, weeds, dead material (kg DM ha^{-1}).

Keywords: dead material, IR, morphological composition, plant height, weeds.

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Introduction

The development of livestock production systems must be oriented towards profitable production, so they must increasingly depend on low-cost and easy-to-handle feeds, and this can be achieved with grasses and legumes to produce perennial or temporary forages of high production and quality (Elizondo and Boschini, 2001).

Sowing density is considered an important factor in crop management, to obtain higher forage yields, the population per area must be increased and a higher leaf:stem ratio by light competition must be achieved, if an optimal density is not achieved, increases in production costs and lower yields can be caused (Solís *et al*., 2007). The use of high population densities in corn translates into better land use (Reta *et al*., 2000; Subedi *et al*., 2006).

The growth analysis is an instrument to know the formation and accumulation of biomass, which helps determine the morphological composition of the crop at each stage of growth, and it is possible to define the yield per hectare of leaves, stems, dead material, which can be managed as indicators of quality of the forage harvested (Rodríguez and Larqué, 1988). For this purpose, the present work had the objective of determining a growth analysis and knowing the accumulated variation of forage of *Lotus corniculatus* L., at different ages and sowing densities.

Like all forages, *Lotus corniculatus* has disadvantages such as slow establishment and low persistence, characteristics that are desirable in the early stages of plant development and throughout their productive cycle, it is essential to allow the recovery of plants (Frame and Laidlaw, 1998; Ixtaina and Mujica, 2010). The quality of forage, palatability and its versatility of use is an important option for livestock feed.

Materials and methods

Locality and date

The research was conducted under field conditions using variety (202700) of *Lotus corniculatus* L., during the spring season from April 12 to May 31 (eight weeks), 2021. In the College of Postgraduates, Montecillo Campus, State of Mexico, at an altitude of 2 250 masl. According to the Köppen climate classification modified by García (2004), the region has a climate C (W0) (W) b (i') g, which corresponds to a temperate subhumid climate with rains in summer and dry season in winter, with little thermal oscillation, an average annual rainfall of 686 mm and an average annual temperature of 15.9 °C, with May being the warmest month and January the coldest month.

Plant material

Original seed of genotype 202700 of *Lotus corniculatus* L., germplasm of erect habit and native to Uruguay, was used, which was obtained through the Plant Genetic Resources Management Program of the College of Postgraduates and the Department of Agriculture of the United States of America (USDA-ARS), in Beltsville, Maryland, USA, which was evaluated for the first time in 1997, in an adaptability trial (García *et al*., 2015; Álvarez *et al*., 2018). But because the plants sown in the field have not generated seed, they continue to be reproduced by means of cuttings taken from the crown of these plants.

Treatments and experimental unit

The experiment was established under a completely randomized design in split plots, two treatments and eight repetitions. Variety 202700 was established using plant material reproduced in pots under greenhouse conditions obtained from mother plants (cuttings taken from the crowns), in December 2019, at two densities (12 000 plants ha^{-1} and 16 000 plants ha^{-1}), they were arranged in strips (3 x 4 m) for each treatment separated at 25 and 20 cm between plants respectively, the first cut was made one week after a uniformization cut at 5 cm and consecutively eight continuous weekly cuts during a regrowth cycle (56 days) in spring; irrigations were scheduled every 15 days, weedings were carried out every month before the first cut, there were no pest problems; however, the presence of white grub was detected, which did not cause damage, no type of fertilizer was applied.

Variables evaluated

Forage yield

To determine the yield of dry matter, per each repetition, a frame of 0.25 m^2 (50 x 50 cm) was randomly placed, the selected fodder was cut 5 cm at ground level, deposited in previously identified paper bags, and dried in a forced air oven, at a temperature of 55 °C for 72 h, up to constant weight. To determine the yield per unit area (kg DM ha^{-1}) (AOAC, 1996), it was weighed on a Truper digital scale (capacity 1-10 kg). The seasonal yield was established as the sum of the forage harvested during the season in spring.

Plant height (PH)

For the average height of the plants, a graduated ruler of 50 cm in length and 1 mm in precision was used, which was placed randomly in each plot, so that the lower part of the graduated ruler (0 cm) was at the ground level. Subsequently, a device that the ruler has was arranged vertically above the plant canopy and slid down, until it touched some morphological component and the height of 5 measurements was recorded. A measurement per repetition was also recorded with the plate, which is calibrated in such a way that it indirectly measures the density of the forage, which compresses and records the height. It consists of resting this tool on the ground and dropping the dish until it touches the plants.

Botanical and morphological composition

To determine the botanical composition, the harvested forage of *L. corniculatus* was used and separated into its morphological components: leaves, stems, weeds and dead material, they were placed in a paper bag and weighed individually on a Truper digital scale with a capacity of 500 g and an approximation of 0.01 g, each component was dried separately in a forced air oven, at a temperature of 55 °C for 72 h to obtain its dry weight (AOAC, 1996). To determine the importance, as a percentage of the harvested forage, of the botanical and morphological component, the following formula was used: formula: PMC= (COMP∗100). Where: PMC= percentage by morphological component (%); COMP= subsample of the separated component; Y= forage yield (kg DM ha^{-1}).

Leaf-stem ratio

The data originated from the morphological composition (leaf and stem) of *L. corniculatus* plants are used to estimate the leaf: stem ratio, which was calculated by the following formula: L:S= L/S. Where: L:S= leaf:stem ratio; L= dry weight of the leaf component (kg DM ha^{-1}); S= dry weight of the stem component (kg DM ha^{-1}).

Average growth rate

The data of forage yield per cut were used to calculate the growth rate (GR) by dividing the quantity of growth of forage harvested weekly by the elapsed time. Using the following formula: GR= FH/t. Where: FH= forage harvested (kg DM ha^{-1}); t= days between one cut and the next.

Intercepted radiation

To perform this measurement, an AccuPAR ceptometer model LP-80 was used, which has sensors that capture photosynthetically active radiation (PAR), distributed homogeneously over a one-meter bar. After calibrating it, the bar was placed at the ground level to make the measurement, which was recorded on a screen. Five measurements per repetition were performed. To obtain the intercepted radiation from the radiometer, the following formula was used: IR= 100-(RG/TR∗100). Where: IR= intercepted radiation (%), TR= total radiation over the canopy (photons); RG= radiation at the ground level (photons).

Statistical analysis

An analysis of variance was performed with the GLM procedure of the statistical program Statistical Analysis System (SAS, 1999), using the model corresponding to the design used. Subsequently, a comparison of means of each dependent variable using the Tukey test ($p< 0.05$) and a regression analysis for each variable were made in order to describe the trend, its coefficient of determination and significance.

Results and discussion

Forage yield

When analyzing the behavior of the variables that were evaluated, significant differences were observed between treatments ($p< 0.05$) for the variables fresh weight and dry weight of stem, leaf, weeds and dead material as a function of the sowing densities. Table 1 shows the comparison of forage yield means by botanical and morphological component of *L. corniculatus* L., of genotype 202700 at different ages, observing that the variables increased steadily, with the density D2 being higher in terms of fresh weight, dry weight, stems, leaves, W and MM, all of them in kg DM ha^{-1}, and the IR (%) than D1, the latter being the one with the highest value for the variable L:S.

Table 1. Forage yield by botanical and morphological component of *Lotus corniculatus* L. genotype 202700, as a function of two sowing densities.

Treat	Fw (kg)	Dw (kg)	S (kg)	L (kg)	L:S (kg)	W (kg)	DM (kg)	IR (%)
D1	17 680 b	3 120 b	146 b	310 b	2.12 a	37 b	14 b	77 b
D2	21 280 a	3 720 a	160 a	321 a	2 b	39 a	17 a	83 a

a, b, c= different letters in the same column are statistically different. Tukey at 0.05. Treat= treatment; D1= 12 000 plants per ha^{-1}; D2= 16 000 plants ha^{-1}; Fw= fresh weight; Dw= dry weight; L-S= leaf-stem ratio; W= weeds; DM= dead material (kg DM ha^{-1}); IR= intercepted radiation.

Botanical and morphological composition

Table 2 shows the comparison of forage yield means by botanical and morphological component of *L. corniculatus*, as a function of eight consecutive weekly cuts, observing that the variables increased steadily and showed statistical differences ($p< 0.05$), the highest results for the variables were the following: for fresh weight they were reached at 42 days (28 060 kg) and 49 days (27 840 kg), dry weight at 42 days (4 882 kg), for stems at 42 days (267 kg) and 56 days (262 kg), leaves at 42 days (428 kg), 49 days (434 kg) and 56 days (439 kg), leaf-stem ratio at seven days (125 kg), 14 days (125 kg) and 21 days (126 kg), for weeds at 42 days (43 kg), dead material at seven days (20 kg) then remained constant at regrowth, and the greatest amount of intercepted radiation (IR) was reached at 49 days (92%). The regression models and coefficients of determination were high ($R^2> 0.96$) in the variables evaluated, the model that best fitted was the polynomial one (Figures 1, 2, 3, 6, 7) for the two treatments.

Table 2. Forage yield by botanical and morphological component of *Lotus corniculatus* L. genotype 202700, as a function of days after cutting.

DAC	Fw (kg)	Dw (kg)	S (kg)	L (kg)	L:S (kg)	W (kg)	DM (kg)	IR (%)
7	2 335 f	800 g	24 g	71 f	2.95 a	37 de	20 a	51 e
14	8 462 e	2 020 f	59 f	182 e	3.08 a	35 e	16 bc	62 d
21	14 510 d	2 775 e	82 e	249 d	3.04 a	35 e	14 de	76 c
28	20 692 c	3 482 d	139 d	326 c	2.35 b	36 de	13 e	88 b
35	26 452 b	4 140 c	208 c	395 b	1.9 bc	40 bc	14 cd	91 a
42	28 060 a	4 510 b	247 b	428 a	1.73 c	43 a	16 b	91 a
49	27 840 a	4 882 a	267 a	434 a	1.63 c	42 ab	16 b	92 a
56	27 425 ab	4 730 ab	262 a	439 a	1.68 c	38 cd	16 b	91 a

a, b, c= different letters in the same column are statistically different. Tukey at 0.05. DAC= days after cutting; Fw= fresh weight; Dw= dry weight; S= stem; L= leaf; L-S= leaf-stem ratio; W= weeds; DM= dead material (kg DM ha^{-1}); IR= intercepted radiation.

Average growth rate

Figure 1 shows the results obtained, which show constant growth, reaching the peak at 42 days for the two densities with a tendency to decrease from the sixth week; Wilson *et al*. (2017), in growth curves of three barley lines, and Rojas *et al*. (2018), in Cobra grass, found a similar behavior and mention that the highest yields are obtained in the sixth week of regrowth and then they tend to decrease, the above is corroborated by the data obtained. Hodgson (1990) mentions that the balance between the growth rate and loss of biomass of a meadow varies in the season of the year, so the knowledge of seasonal changes in the growth curve allows establishing the frequency of defoliation at which they must be harvested to obtain a high yield of good quality forage.

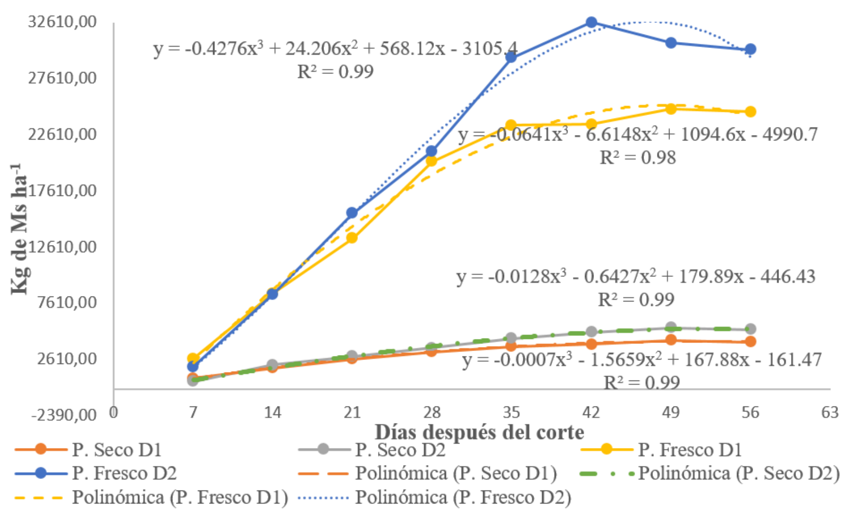

Figure 1. Changes in growth rate over time (56 days) of bird's-foot trefoil (*Lotus corniculatus* L.) and dry matter yield at 2 sowing densities. D1= 12 000 plants ha^{-1}; D2= 16 000 plants ha^{-1}.

Calzada *et al*. (2014) mention that the growth rate of maralfalfa grass was determined by presenting a constant increase, from germination to obtain a maximum at 151 days, decreasing from that moment. Beltrán *et al*. (2005) found that the higher growth rate is related to the higher amount of root and that it decreases as a result of the gradual increase in the senescence rate and the reduction in the net photosynthesis rate per unit area.

Changes in forage accumulation

The curves of growth rate and dry matter yield of *L. corniculatus* L., at different sowing densities D1: 12 000 plants ha^{-1} and D2: 16 000 plants ha^{-1}, the maximum development is observed at 49 and 42 days respectively (Figure 1). The dynamics of forage accumulation (Figure 2) in stem 245.6 and 288.5 kg DM ha^{-1} at 49 days for D1 and D2 respectively, for the variable leaf 429.35 kg DM ha^{-1} at 56 days for D1 and 451.5 kg DM ha^{-1} at 42 days for D2.

Growth increased progressively as the age of regrowth progressed in the total plant yield curve (Figure 3). The highest accumulation of dry matter for D1 with 4 315 kg DM ha^{-1} (Figure 4) and D2 was obtained at 49 days with 5 450 kg DM ha^{-1} (Figure 5). In the case of leaf, the maximum yield was 429.35 and 449.15 kg DM ha^{-1} for D1 and D2, as for stem yield the same behavior was observed 245.60 and 288.50 kg DM ha^{-1} respectively (Figures 2, 3 and 4). With respect to the components, stem and dead material (Figures 4 and 5), it is observed that they are ordered positively with the development of plants, which agrees with other studies Zebadúa *et al*. (2001); Lafarge and Loiseau. (2002); Amaro *et al*. (2004); Calzada *et al*. (2014); Calzada *et al*. (2018); Gutierrez *et al*. (2018); Rojas *et al*. (2019) mention that stem production is a very significant key factor in meadow support, which highlights the importance of production monitoring.

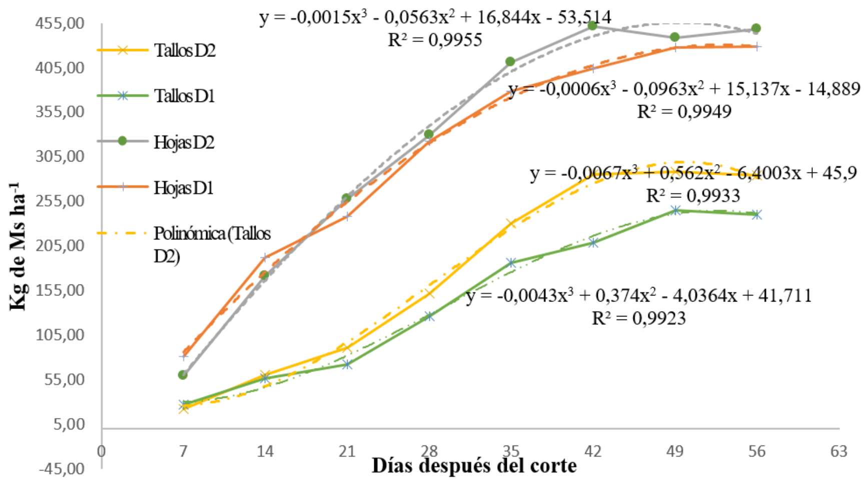

Figure 2. Dynamics of forage accumulation in stems and leaves of bird's-foot trefoil (*Lotus corniculatus* L.) in 2 sowing densities in a 56-day growth cycle. D1= 12 000 plants ha^{-1}; D2= 16 000 plants ha^{-1}.

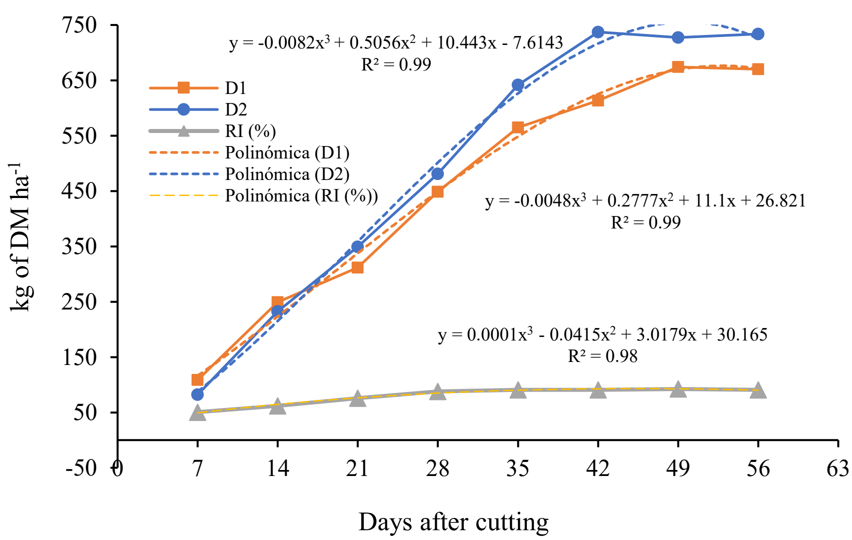

Figure 3. Total yield of bird's-foot trefoil (*Lotus corniculatus* L.) in a meadow with 2 sowing densities in a 56-day growth cycle. D1= 12 000 plants ha^{-1}; D2= 16 000 plants ha^{-1}; RI= intercepted radiation.

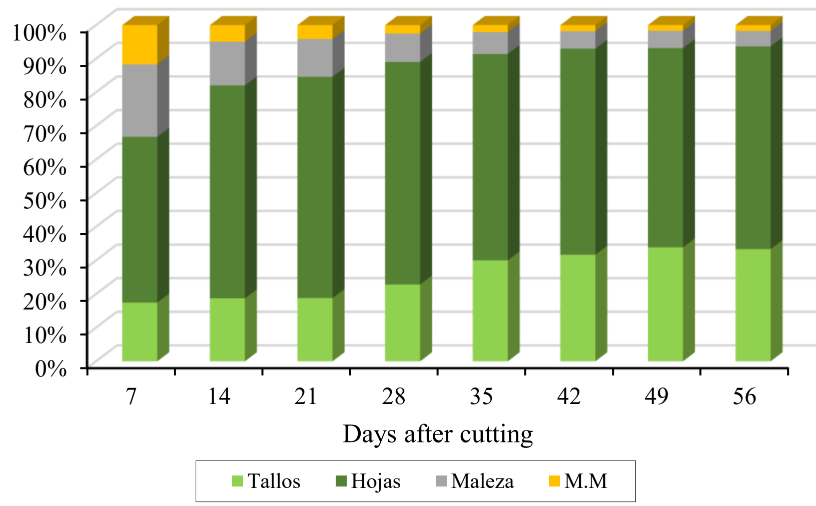

Figure 4. Changes in botanical and morphological composition in a meadow of bird's-foot trefoil (*Lotus corniculatus* L.) with a sowing density of 12 000 plants per ha^{-1} in a 56-day growth cycle. M.M= dead material.

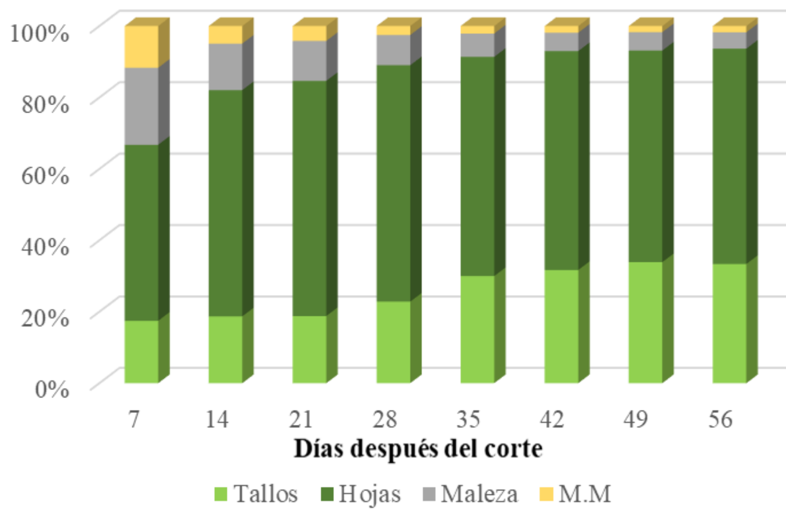

Figure 5. Changes in botanical and morphological composition in a meadow of bird's-foot trefoil (*Lotus corniculatus* L.) with a sowing density of 16 000 plants per ha^{-1} in a 56-day growth cycle. M.M= dead material.

The highest leaf yields, combined with the highest L:S ratio, the peak was reached at 21 days after cutting for both densities with 113.84 kg DM ha^{-1} for D1 and 131.08 kg DM ha^{-1} for D2, the accumulation of dry matter in leaf was higher than that of stem, after that age, the ratio began to reverse up to 56 days (Figure 6).

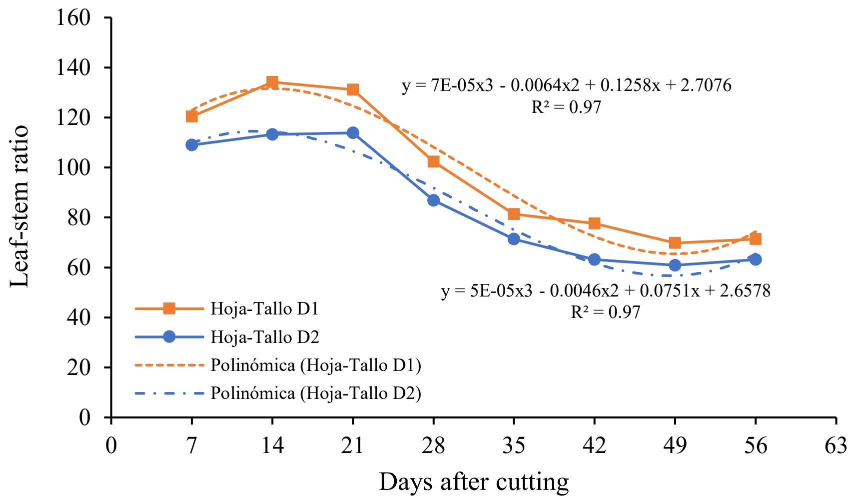

Figure 6. Changes over time in the leaf-stem ratio of bird's-foot trefoil (*Lotus corniculatus* L.). D1= 12 000 plants per ha^{-1}; D2= 16 000 plants per ha^{-1}.

Plant height (PH)

Regarding this variable, at the first exploitation, there were changes over time (Figure 7) with the two methods used, with the rule method the highest data were obtained for D1 at 49 days with 23.58 cm and 32.03 cm for D2 at 56 days, while with the plate method higher data were obtained for D1 with 20.94 cm and 32.81 cm for D2 at 56 days. Similar results are reported by García *et al*. (2015); Álvarez *et al*. (2018) when evaluating different *L. Corniculatus* genotypes in the same study area.

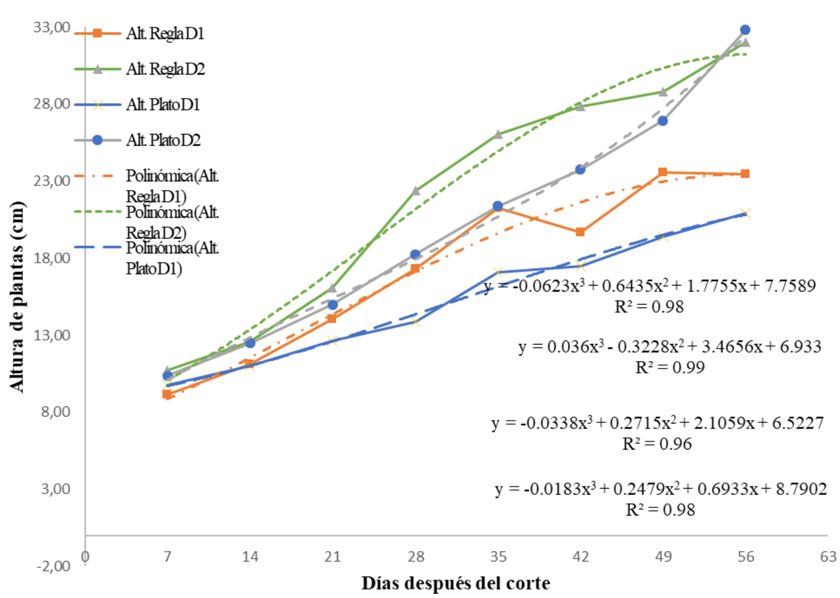

Figure 7. Changes over time in height of bird's-foot trefoil (*Lotus corniculatus* L.). Alt.= height; D1= 12 000 plants ha^{-1}; D2= 16 000 plants ha^{-1}.

Intercepted radiation

Tables 1 and 2 show the average results of intercepted radiation (%) by treatment 77% for D1 and 83% for D2 and between cuts for each week (Figure 3) 51, 62, 76, 88, 91, 91, 92, 91% respectively, reaching the peak at 49 days. Figures 4 and 5 show the average yields by component, observing that the leaf was the one that contributed the most to the yield (60.87%), followed by stem (25.84%), weeds (9.46%), DM (3.83%) for D1 and for D2, leaf (57.41%) followed by stem with (28.70%), weeds (9.41%), DM (4.49%).

In works carried out in tropical and temperate grasses, Hodgson (1990); Da Silva and Hernández (2010); Wilson *et al*. (2018) mention that the optimum point of harvest of plants is when reaching 95% of light interception, where there is little accumulation of dead material and greater amount of leaf. In oats, Wilson *et al*. (2017) did not find the maximum intercepted radiation expected due to environmental conditions and possibly because higher sowing densities are required. Juskiw *et al*. (2000) found that sowing density, sowing method, and sampling date have a significant effect on the number of botanical and morphological variables, so possibly some of these variables or their combination did not allow reaching 95% of IR, data that, in this work, did not reach that point (92%).

Conclusions

Of the yield components, the leaf contributed the most to the forage yield in the spring season for the two densities evaluated. The accumulation of forage and the components stem, weeds and dead material are positively related to the maturity of the plant and the increase in dry matter was proportional to the age of regrowth, reaching the maximum yield for the spring season at 49 days.

As for intercepted radiation, at the peak 92% was obtained at 49 days. The height of the plants behaved positively as the meadow developed, noting changes in relation to sowing density. The density with the best behavior in general was D2, so it is concluded that *Lotus corniculatus* L. is a material that has a good forage aptitude in terms of dry matter yield and number of leaves. It is recommended to continue these studies using longer evaluation periods to determine an annual growth analysis.

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