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Article

Alfalfa (*Medicago sativa* L.) biomass yield at different pasture ages and cutting frequencies

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Abstract:

Cutting frequency and pasture age are strategic variables in defining alfalfa crop management aimed at increasing biomass yield. An analysis was done to identify the effects of three cutting frequencies (three, four and five weeks) in the spring-summer cycle on dry matter production, growth rate and performance variables in alfalfa (*Medicago sativa* L. Oaxaca criolla) in three pasture ages (one, two and three years). A random block design with a 3x3 factorial arrangement (cutting frequency and pasture age) was used. Highest (P<0.01) average dry matter yield (7,528 kg DM ha^{-1}) and growth rate (257 kg DM ha^{-1} d^{-1}) were recorded at the one-year pasture age. Average dry matter yield was highest at the four-week cutting frequency (6,844 kg DM ha^{-1}), which was 29% higher than at three weeks and 16% higher than at five weeks. In the one-year pasture, leaf and stem production was 45% higher than in the three-year pasture and forage height was 32% higher. At the four-week cutting frequency leaf production was 21% higher than at the three-week frequency, while stem production was 49% higher and forage height was 33% higher. The evaluated variables and their interactions determined estimated alfalfa component yield.

Key words: Alfalfa, Cutting frequency, Pasture age.

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Introduction

Factors such as forage yield, protein content, digestibility, rusticity and adaptability to different environmental conditions have made alfalfa (*Medicago sativa* L.) the most cultivated Fabaceae worldwide. It is ubiquitous as an animal feed, and is even recommended as a dietary supplement to combat malnutrition and digestive disorders in humans(1). In 2016, alfalfa was grown on 387,154 ha in Mexico, with an average annual yield of 86 t per hectare green forage. In the state of Oaxaca, 3,489 ha are sown in alfalfa the production from which accounts for 1.45% of national production, placing the state sixteenth nationwide. The majority (95%) of alfalfa production in Oaxaca occurs in the central valleys, where it is the second largest crop(2).

Pasture yield, growth and persistence, as well as forage quality, depend on cut frequency and intensity, and season(3,4). Cut frequency determines forage nutritional value and morphogenesis. Defining a management plan based on biomass accumulation rate is therefore fundamental to alfalfa production(5,6). Pasture regrowth age, or fallow time, affects animal production profitability, particularly in milk production systems(7). Cut frequency also modifies regrowth mortality rate and survival by allowing radiation to reach the crown level, which affects resprout rate and stem death, as well as photosynthesis in early post-cut foliage(8).

Biomass accumulation rate determines cut frequency and therefore time to pasture harvest. The leaf:stem ratio, and consequently forage quality, varies with plant maturity, recovery time between successive cuts, regrowth time, season and environmental conditions[8-11]. Forage nutritional value is also correlated to other phenological variables such as weight, density and stem size and leaf count per stem[10,12,13].

The physical and chemical changes in forage caused by pasture age and cut frequency manifest in variations in digestibility, yield, and lignin, fiber and protein contents[14,15]. A species' potential forage production also depends on the morphological structures (leaf and stem) remaining post-cut and root carbohydrate reserves for production of new stems and leaves[16].

In a previous study evaluating alfalfa pastures over five consecutive years average annual forage yield was highest (18,300 kg ha^{-1}) at two and four years, independent of initial sowing density[17]. Another study evaluating nineteen alfalfa varieties over two consecutive years found differences ($P < 0.05$) in plant height, main stem diameter, green forage and dry matter production, and protein content[18].

The present study objective was to evaluate forage yield, leaf and stem production, crop growth rate and the leaf:stem ratio in alfalfa (*Medicago sativa* L., var. Oaxaca criolla) at different pasture ages and cut frequencies.

Materials and methods

Data were collected during spring-summer (March - October 2013) from alfalfa (*Medicago sativa* L., var. Oaxaca criolla) pastures under cultivation for one, two and three years at the experimental agricultural field of the Technological Institute of the Valley of Oaxaca, Nazareno Xoxocotlan, Oaxaca (17°01' 20.40" N; 96°44'51.50" W; 1,530 m asl). The predominant climate in the region is dry steppe (BS_1h, (h)), with a 20.6 °C average annual temperature, and 645 mm average annual precipitation[2]. Average monthly precipitation and temperature during the study period were within normal values (Table 1).

Table 1: Average monthly temperature and precipitation during study period (March – October 2013).

Month	Temperature (ºC)			Precipitation (mm)
	Max.	Min.	Aver.	Means
March	31.0	9.4	35.8	2.8
April	30.2	10.4	35.5	2.7
May	30.3	12.3	36.4	2.7
June	28.9	15.1	36.4	4.9
July	29.7	13.0	36.2	3.6
August	27.0	13.0	33.5	3.1
September	26.8	15.2	34.4	8.3
October	26.8	13.0	33.3	1.6

An experimental design of random blocks was implemented based on terrain slope, establishing 36 experimental units of 9 m^2 each. The 3 x 3 factorial arrangement included the factors pasture age (one, two and three years since sowing) and cut frequency (three, four and five weeks), generating nine treatments. The pastures were not fertilized and were sprinkler irrigated to field capacity.

A homogenizing initial cut to 5 cm was done at the beginning of the experiment to reduce the covariate effect. Forage yield per cut in each experimental unit was measured by randomly placing a steel frame (0.25 m^2) on an area in which all forage in the frame was cut to 5 cm(19). Harvested biomass was stored in paper bags marked with the treatment number and replicate, and dried in a forced air oven at 55 °C for 72 h to constant weight to determine dry matter content.

Forage height was recorded before each cut with a 1-meter-long graduated ruler with 0.5 cm accuracy. Ten height measurements were taken inside each experimental unit on randomly selected plants with the ruler placed vertically from plant base to apex(20,21).

The harvested forage samples from each experimental unit were homogenized and a subsample of approximately 25% taken. This was separated into morphological components (stems, leaves, inflorescence and dead matter) and the weight of each measured in dry base(4).

Growth rate (GR) was calculated using dry matter yield per cut with the following formula:

$$GR = \frac{HF}{t}$$

Where:

HF = harvested forage (kg DM ha^{-1}) and

t = days from one cut to the next.

The leaf:stem ratio per cut was calculated by dividing average weight of leaves by average weight of stems.

Values were grouped and analyzed with the PROC MIXED procedure in the SAS® statistics software package[22]. The Akaike criterion was used to select the variance and covariance matrix[23]. This in turn was used to identify the effects of the sources of variation (cut frequency: 3, 4 and 5 weeks), pasture age (one, two and three years), which were considered fixed effects while the block effect was treated as random[24]. Treatment means were estimated using LSMEANS, and comparison between them done with the probability of difference (PDIFF) using a 5% significance level. For the linear regression between pasture height and forage yield, the variables considered were forage height and dry matter yield. Linear regression equations were produced using 95% confidence intervals, and yield predicted by pasture age and cut frequency (CF). These calculations were done using a linear polynomial model in the Wizard Regression module of the SigmaPlot ver. 10 package[25].

Results and discussion

The factorial analysis showed that average biomass production was highest at the four-week CF (6,844 Kg DM ha^{-1}) and in the one-year pasture (7,528 Kg DM ha^{-1})(Table 2). These levels were 42% higher than in the two-year pasture and 44% higher than in the three-year pasture. Interaction between the factors was highly significant (P<0.001).

Table 2: Forage, stem and leaf yields, height, growth rate and leaf:stem (L:S) ratio at the studied pasture ages and cut frequencies

Factor		Forage yield kg DM ha^{-1}	Leaf yield kg DM ha^{-1}	Stem yield kg DM ha^{-1}	Height cm	Growth rate kg MS ha^{-1}d^{-1}	Ratio L:S
Pasture age (years)	1	7528 a	5105 a	2424 a	37 a	275 a	2.6 b
	2	5290 b	3321 b	1969 b	30 b	200 b	3.4 a
	3	5208 b	3511 b	1669 c	28 b	194 b	2.6 b
	MSE	286 **	170 **	133 **	1.4 **	13 **	0.11 **
Cut frequency (weeks)	3	5289 c	3697 b	1592 c	27 c	252 a	2.8 a
	4	6844 a	4474 a	2370 a	36 a	244 a	2.4 b
	5	5892 b	3766 b	2127 b	32 b	173 b	2.3 b
	MSE	354 **	243 **	129 **	1.5 **	13 **	0.1 **

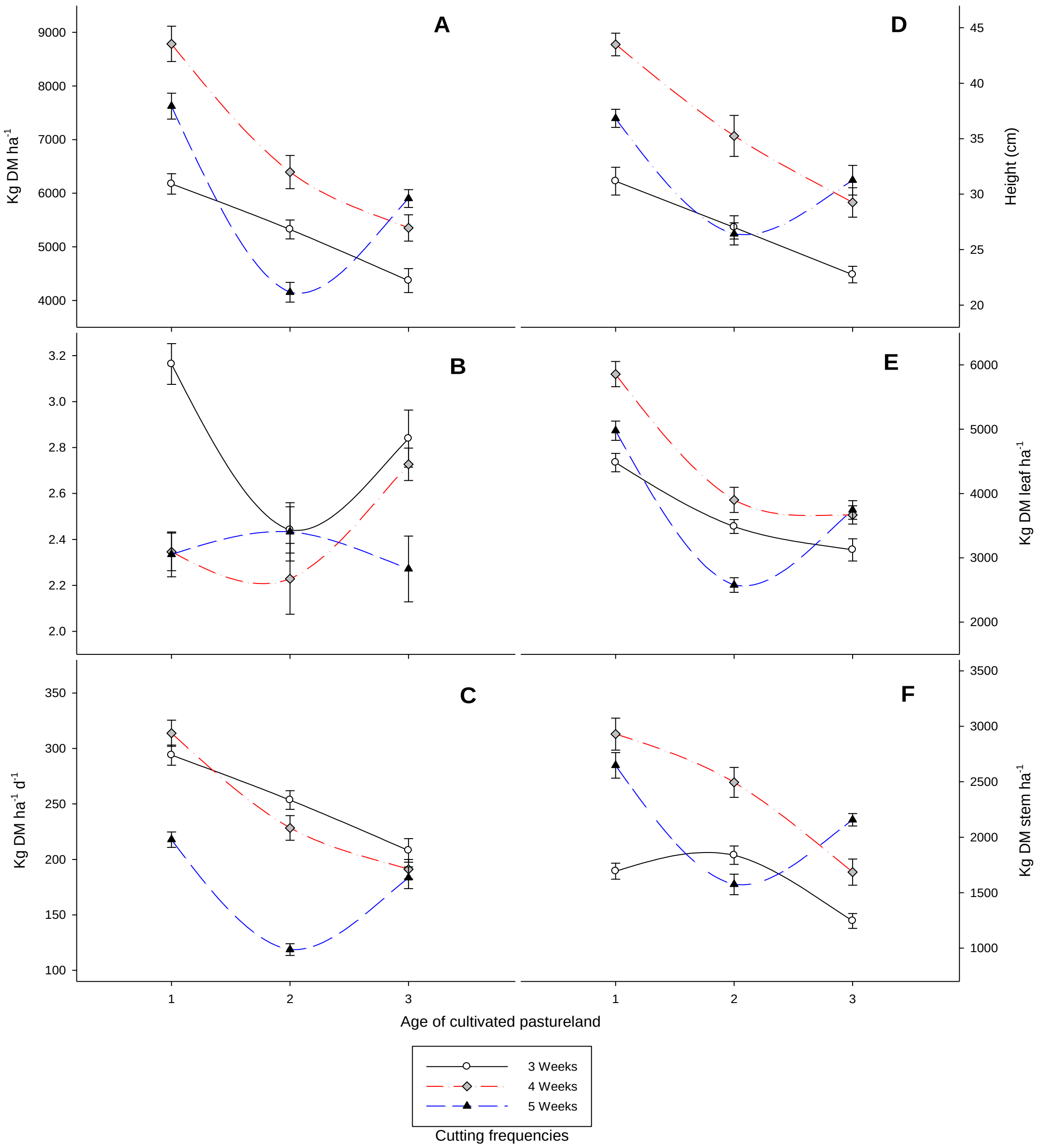
Pasture age/cut frequency interaction was highly significant (*P*<0.001).

a,b,c Different lowercase case letters in the same column indicate significant difference (Tukey 0.05).
MSE = mean standard error.

** (*P*<0.05).

When run by treatment, the four-week CF produced the highest (*P*<0.01) dry matter yields in the one-year (8,786 kg DM ha^{-1}) and two-year (6,394 kg) pastures (Figure 1A). Yield in the one-year pasture at the four-week CF was 42 % higher than the three-week CF and 20 % higher than the five-week CF. Yield in the two-year pasture at the four-week CF was 15 % higher than in the three-week CF and 54 % higher than in the five-week CF. However, biomass accumulation was highest (*P*< 0.01) in the three-year pasture at the five-week CF; this was 35 % higher than the three-week CF at the same pasture age. The latter also had the lowest biomass accumulation value among the one- and three-year pasture ages, suggesting that older pastures require longer inter-cut rest periods.

Figure 1: Dry matter yield (A), leaf:stem ratio (B), crop growth rate (C), pasture height (D), leaf yield (E) and stem yield (F) in alfalfa pastures at different cut frequencies and pasture ages



In the factorial analysis, leaf yield was highest in the one-year pasture (5,105 kg DM ha^{-1}); this is 53 % higher than in the two-year pasture and 45 % higher than in the three-year pasture. Among the cut frequencies, this parameter was highest at the four-week CF (4,474 kg DM leaf ha^{-1}) (Table 2). When analyzed by treatment, the highest leaf yields were recorded at the four-week CF in the one- (5,856 kg DM leaf ha^{-1}) and two-year pastures (3,900 kg). These levels were 30% higher than in the three-week CF and 51 %

higher than in the five-week CF. The three-week CF produced the lowest leaf yield in the one- and three-year pastures (Figure 1E).

Factorial analysis showed the highest stem yields (Table 2) to be in the one-year pasture (2,424 kg DM ha^{-1}) and at the four-week CF (2,370 kg). Among the treatments, the four-week CF produced the highest leaf yield (P<0.01) in one- and two-year pastures, with an average of 2,710 kg and an average contribution of 34% of total yield (Figure 1F). The five-week CF produced the highest leaf yield in the three-year pasture (2,157 kg), which represented 37 % of total yield.

The crop growth rate coefficient was lowest, with no differences between treatments (P>0.05), in the two- and three-year pastures (Table 2). The highest coefficients were at the three- and four-week CF (Figure 1C), with no differences between them.

The present results agree with a study evaluating the growth dynamic of three-year old alfalfa (var. Oaxaca criolla) which concluded that in spring and summer cuts should be done at week four or five to obtain the highest leaf proportions and the least amount of senescent material(6). This supports the hypothesis that environmental conditions (temperature and precipitation) during the spring-summer period promote higher leaf and forage production.

A report evaluating growth dynamics in two alfalfa varieties found that age at resprouting affected forage yield at different times of the year, and proposed that cuts should be made at week four in the summer and week six in the spring(5). Another study addressing growth in five alfalfa varieties found that yields were highest in the summer using a four week CF, and that the accumulated production in spring and summer represented 58% of total annual production(3). Overall, the above reports suggest that, independent of variety, post-cut pasture recovery period in different seasons is the variable which determines yield and component proportions.

The importance of season (spring-summer) is supported by a study of a three-year-old alfalfa pasture in which 57 % of forage production occurred in spring and summer(4). No differences (P>0.05) in yield were observed between three-, four- and five-week CF in the spring, which differs from the present results. This discrepancy could be due differences in experimental sites since climatic conditions differed because of geographical location. The same study also found that forage production in summer did not differ (P>0.05) between the four- and five-week CF, although both these CF produced values higher (P<0.05) than the three-week CF(4). This coincides with the present results in the three-year pasture, and further supports the hypothesis that alfalfa crop age influences reactions to inter-cut recovery periods.

In an evaluation of four-, five- and six-week CF in a pasture shortly after planting and in two consecutive years, no differences (P>0.05) in forage yield were observed, although yield was 1.46 % higher in the first year than the second year(26). However, differences (P<0.05) were present between CF within the same year, with the four-week interval resulting in the lowest yield (6,600 kg DM ha^{-1}). Again, discrepancies between this study and the present results can be attributed to their being done in different geographical locations.

In another study, four alfalfa varieties in newly planted pastures and subjected to severe (28 d) and light (35 d) CF found that in the summer all four varieties yielded 24 % more (P<0.05) forage when cut every 28 d and 70 % more (P<0.05) when cut every 35 d(27). However, the 28-d CF resulted in a better leaf:stem ratio than the 35-d CF. This study agrees with the present results for the one- and two-year pastures, but differs from those for the three-year pasture. Pasture age is apparently a determining factor in the leaf:stem ratio.

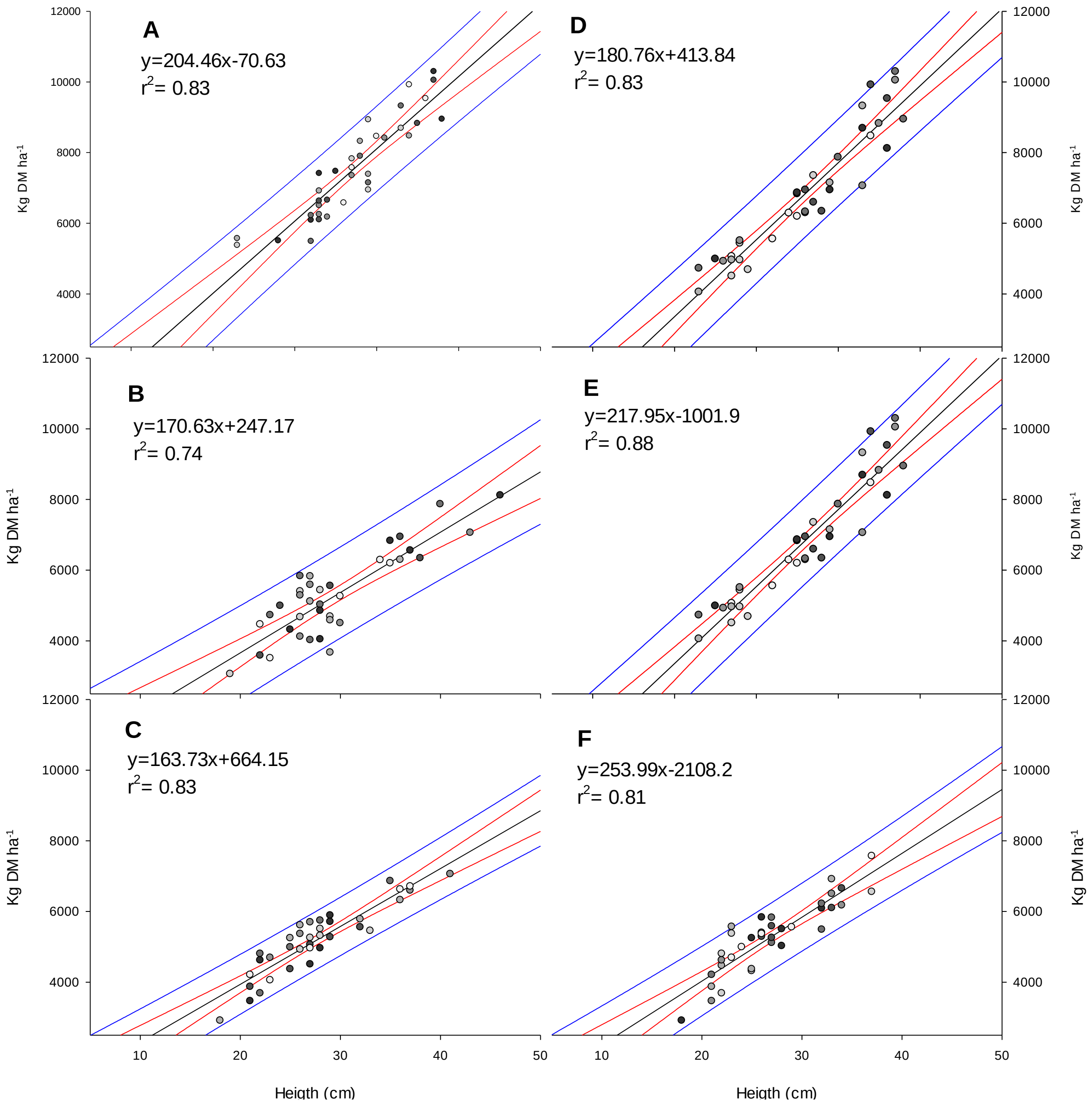
An evaluation of accumulated alfalfa yield over five consecutive years found that yield was highest in the two- and four-year pastures (18,300 kg ha^{-1}, average), independent of initial planting density(17). This contrasts with the present results, and again suggests that climatic conditions may have affected biomass accumulation in the years evaluated.

Forage height was highest in the four-week CF (36 cm), and the one-year pasture (37 cm) (Table 2). It was lowest (28 cm) in the three-year pasture. Analysis by treatment (Figure 1D) showed that forage height was lowest with the three-week CF in the one- (31 cm) and three-year pastures (23 cm). The five-week CF was highest (P<0.01) in the three-year pasture (31 cm). In a previous study average forage height only varied (P>0.05) during the first year after planting, independent of alfalfa variety(28). However, other factors such as ambient temperature can also influence plant growth performance and height. A different study evaluating nineteen alfalfa varieties reported differences in plant height, main stem diameter, green forage and dry matter production, and protein content during two consecutive years after planting(18). Considering the above it appears that under certain experimental conditions crop variety can influence growth and yield parameters.

The leaf:stem ratio did not differ (P>0.05) between the one- and three-year pastures, nor between the four- and five-week CFs (Table 2). Both the four- and five-week CFs had lower values than all the pasture ages (Figure 1B). Leaf proportion was highest (P<0.01) at the three-week CF (2.8) and in the two-year pasture (3.4) (Table 2). Alfalfa varieties that produce high dry matter yield also have a low leaf:stem ratio(3), highlighting the importance of evaluating stem weight and height in relation to leaves. As plant height increases the leaf proportion decreases, a phenomenon clearly linked to phenological stage. Some studies have shown that the leaf:stem ratio decreases beginning in the preflowering stage, a trend that becomes particularly notable in the initial flowering stages(29). Growth, and thus leaf:stem ratio values, can also respond to season. In a study evaluating alfalfa physiological condition using mean stage by count (MSC) and mean stage by weight (MSW), values were clearly lower (P<0.001) in the autumn than in the spring-summer even at equal CFs, indicating that plant growth is not uniform year round(30).

The linear regressions showed the coefficient of determination to be higher than 0.8 for all variables (Figures 2A, 2C, 2D, 2E and 2F), except the two-year pasture (r^2 = 0.74, Figure 2B). In practical terms the equations indicate that an increase of one centimeter in the biomass represented 204.4 kg DM ha^{-1} in the one-year pasture, 170.7 kg in the two-year pasture and 163.7 kg in the three-year pasture. This same increase represented 180.7 kg at the three-week CF, 217.95 kg at the four-week CF and 253.9 kg at the five-week CF. These results generally coincide with previous reports that state pasture height to have a 0.80+ correlation in C3 forage species[20,21].

Figure 2: Linear regressions between forage height and yield in one- (A), two- (B) and three-year (C) pastures, and at three- (D), four- (E) and five-week cutting frequencies



Straight slope (black line), confidence interval (0.95) (blue line), predicted data (red line).

More frequent cutting reduces N mobility in roots, consequently lowering the photosynthetic capacity of newly emerged leaves post-cut, leading to lower foliar expansion rates and less forage yield[13]. This effect can be observed in the stem appearance rate, the number of sprouts per plant, the leaf area index and the stem death rate[8].

The results observed in the treatments evaluated in the present study may be partially explained by the quantity of reserve substances stored in the alfalfa plant crowns and roots[11]. However, longer inter-cut rest periods will not necessarily exponentially increase biomass production. This highlights the need to identify optimum cutting frequency for each forage species, time of year and production condition.

Conclusions and implications

In the studied alfalfa variety (Oaxaca criolla) biomass accumulation was highest in the one-year pasture, with significant relative decreases in the two- and three-year pastures. Using the four-week cut frequency, more biomass production was observed in the one- and two-year pastures. Plant yield proportions were mainly influenced by the interaction between the evaluated factors, resulting in a more complex reaction.

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