Technical Note

## Yield of forage and its components in alfalfa varieties of the Mexican high plateau

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## Abstract:

Alfalfa (*Medicago sativa* L.) is the cultivated legume that is mostly used in milk and meat production in Mexico, due to its high yield and nutritional value. The objective of this study was to evaluate the yield of forage and its components, in five alfalfa varieties, with seasonally defined cutting intervals. The Aragon, Valenciana, Chipilo, Milenia and Oaxaca varieties were randomly distributed in 20 experimental, 12 x 9 m plots, according to a randomized complete block design with four repeats. The evaluations included forage yield in a dry base, stem weight, stem population  $m^{-2}$ , plant population  $m^{-2}$ , leaf:stem ratio, botanical and morphological composition. The highest

and lowest yields were obtained in the Milenia and Aragon varieties, with 20,643 and 14,488 kg DM ha<sup>-1</sup>. The weight per stem was better in Aragon, Chipilo and Milenia, and lower in Valenciana and Oaxaca. Aragon had the greatest stem density, with 634 stems m<sup>-2</sup>, and Oaxaca, with 512 stems m<sup>-2</sup>, had the lowest density. The highest leaf:stem ratio was found in Aragon, with 1.31, and the lowest was found in Oaxaca, with 1.13. In fall and winter, a larger amount of leaves was obtained, independently of the variety, and in summer, there was an increase in all weed varieties. Seasonality was related to yield, with a greater production in spring and summer, due to temperature, and to a greater stem weight. The variety with greatest dry matter yield was Milenia, and Aragon had the lowest yield.

Key words: Forage yield, leaf:stem ratio, stem population.

Received: 18/09/2017

Accepted: 07/05/2018

Alfalfa (*Medicago sativa* L.) has great importance due to its high yield per surface unit and its forage nutritional value<sup>(1)</sup>, and because it is appealing to diverse animals when consumed fresh, as hay or as ensilage<sup>(2)</sup>. Alfalfa is also used to improve vegetal covers, avoid soil erosion, prevent the degradation of prairies and to support sustainability in agriculture and cattle raising activities<sup>(3)</sup>. When this legume is associated with a grain, the prairie's production increases, seasonality is minimized, the nutritional value improves and production costs are reduced, compared to balanced diets<sup>(4)</sup>.

Researchers<sup>(5,6)</sup> showed that the fequency and intensity of alfalfa cutting must be defined based on the state of the plant's development and the season. These parameters are important to achieve balance between quantity, quality and the prairie's persistence<sup>(7)</sup>. It has been observed that alfalfa yield is greater in spring-summer and lower in fall and winter<sup>(8,9)</sup>. Villegas *et al*<sup>(10)</sup> also reported that forage yield of the alfalfa varieties was higher in spring, followed by that of winter and summer, and the lowest yield was recorded in the fall. Idris and Adam<sup>(11)</sup> obtained a higher and lower annual yield in the Hagazi and Cuf 101variety, with harvest frequencies of 25 and 30 d, respectively.

Stem population density and weight have been evaluated in several parts of the world, since they are forage production indicators <sup>(12)</sup>. In an investigation carried out by Chen *et al* <sup>(3)</sup>, an increase in the alfalfa cutting frequency was strongly linked to stem density, increasing until it reached a point of decline, independently of the variety and year of evaluation, with 645, 734 and 688 stems m<sup>-2</sup>, at a frequency of 30, 40 and 50 d. These same authors observed the lower and higher weight per stem, with 0.27 and 0.45 g for the lower and higher frequencies, respectively; this was related to

yield. Other investigations<sup>(13)</sup> mention a strong correlation between a greater weight per stem and higher yield and temperature. Some authors<sup>(14)</sup> report the highest alfalfa yield with a density of 25 plants m<sup>-2</sup>. Morales *et a*<sup>(15)</sup> found a high leaf:stem ratio in 14 alfalfa varieties, with the highest total yield, growth rate and stem density. They also stated<sup>(5)</sup>, that a greater percentage of leaves was obtained in winter, with an average 65 %, and this percentage was lowest in spring. Nevertheless, in Mexico, there is scarce information on these production parameters.

The objective of this study was to evaluate the yield components of five commercial alfalfa varieties, at seasonally defined cutting intervals, with the following attributes: forage yield, weight per stem, stem density, plant density, leaf:stem ratio, botanical and morphological composition.

The study was carried out in the experimental field of the *Colegio de Postgraduados*, in Montecillo, Texcoco, State of Mexico (19° 29' N and 98° 53' W, at an altitude of 2,240 m) from June, 2010 to June, 2011. The climate is temperate sub-humid, the dryest of the sub-humid climates, with a mean annual precipitation of 636.5 mm; rainy season in summer (June to October) and an annual average temperature of 15.2 °C<sup>(16)</sup>. The soil is a Typic Udipsamments, with a sandy loam texture, a pH between 7 and 8 and 2.4 % organic matter<sup>(17)</sup>. Five commercial alfalfa varieties were used: Aragon, Valenciana, Chipilo, Milenia and Oaxaca, sown by broadcast on April 18, 2008. The sown density was 30 kg ha<sup>-1</sup> of pure live seed, adjusted by the germination percentage of each variety.

The study area was divided into 20 plots covering 108 m<sup>2</sup> (12 x 9 m). At the beginning of the experiment (June 2, 2010) standardization cutting was carried out with a tractor-mounted pruning machine, at an average height of 5cm; the experimental phase concluded on June 21, 2011. The cutting interval varied according to the season: in spring and summer the plants were cut every 4 wk in fall, every 5 wk and in winter, every 6 wk, according to recommendations of Mendoza *et al*<sup>(5)</sup>. The prairies were not fertilized and in the seasons with minimal rainfall, these were irrigated at field capacity every two weeks.

In order to evaluate forage yield for each variety, at the start of the study, two fixed squares of  $0.25m^{-2}$  per repeat, were randomly placed. The forage present within each square was harvested one day before cutting, at a height of 5 cm; it was placed in labeled paper bags and dried in a forced air oven, until reaching constant weight. Once dry, the sample's weight was recorded to estimate the dry matter yield per surface unit (kg DM ha<sup>-1</sup>). One day before cutting, 10 stems were randomly cut in each treatment and repeat at ground level, and dried in a forced air oven, until reaching constant weight per stem was calculated. At the beginning of the experiment, two fixed 20 x 20 cm squares were randomly placed in each experimental unit; stems present in each square were counted monthly and later on the average number per season was calculated.

When the experiment began, a  $1 \text{ m}^2$  fixed square was placed in an experimental box, at ground level, where the alfalfa plants were counted on a monthly basis, and changes in population density were recorded, averaging them seasonally.

The leaf:stem ratio was calculated dividing the dry weight of each fraction (leaf/stem), expressed in kg ha<sup>-1</sup>, obtained from the subsample used to estimate the botanical and morphological composition. To obtain the botanical composition, one day before each cutting, a subsample was taken of approximately 20 % of the forage samples harvested to estimate the yield, and each subsample was separated into alfalfa and weeds. Each component was dried in a forced air oven, until reaching a constant weight, and its dry weight was recorded; subsequently, the seasonal yields were averaged.

Data including maximum and minimum temperatures and rainfall accumulated during the study period, were obtained from the agro-meteorological station of the *Colegio de Postgraduados*, located 100m from the experimental area (Figure 1). The maximum temperature was observed in July, 2010 and between March and June, 2011, with an average of 28 ° C, corresponding mainly to spring and summer. The minimal recorded temperature was in December, 2010 and in January and February, 2011, with an average -1 °C, corresponding to winter. The greatest precipitation (mm) was in July, August, Sept and Nov, 2010 and in June, 2011, with an accumulation of 404 mm; this mainly represented the summer and fall seasons.

In winter and spring, the plants were irrigated to field capacity every 15 d. The effect of the studied factors on the response variables was evaluated through an analysis of variance (ANOVA) using the mixed models<sup>(18)</sup> procedure, with a randomized complete block design with four repeats. The comparison of means was done using the Tukey test (P= 0.05).





No significant interactions were found (P>0.05) between the studied factors. In general, average annual contribution to yield was: summer 3 5%, spring 28 %, fall 24 % and winter 13 %. Average forage yield for the alfalfa varieties decreased in the fall (P<0.05) with respect to summer records; also, winter yield was lower (P<0.05) than what was obtained during the other three seasons (Table 1). These results coincide with the higher temperatures recorded in spring-summer (Figure 1), which favored alfalfa development<sup>(19)</sup>, since the optimal temperature for alfalfa growth fluctuates between 15 and 25 °C. On the other hand, dry matter yield for the Milenia variety only was higher (P<0.05) than that of the Aragon variety (Table 1). Villegas *et al*<sup>(10)</sup> report similar yields in the Oaxaca and Valenciana varieties, in this evaluation (21,600 and 20,000 kg DM ha<sup>-1</sup>). However, independently of the alfalfa variety and cutting frequency, the average annual yield<sup>(11)</sup> was 10,552 kg DM ha<sup>-1</sup>.

Variety	Summer	Fall	Winter	Spring	SEM	Annual
Aragon	5188 Ba	3334 Bb	1717 Bc	4248 Aab	456	14488 B
Valenciana	6407 Aa	4093 Ab	2035 ABc	4293 Ab	398	16828 AB
Chipilo	6162 ABa	4386 Ab	2412 ABc	5072 Aab	402	18034 AB
Milenia	7148 Aa	4898 Ab	2776 Ac	5819 Aab	434	20643 A
Oaxaca	6298 ABa	4512 Ab	2217 ABc	4911 Aab	521	17939 AB
SEM	345	432	355	456		897
Average	6241 a	4244 b	2231 c	4869 ab		

Table 1: Seasonal and annual	yield	(kg DM ha <sup>-1</sup> )	) of alfalfa varieties
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abc= means with the same lowercase letter in the same row are not different (P>0.05). ABC= means with the same uppercase letter in the same column, are not different (P>0.05). SEM= Standard error of the mean.

Other researchers<sup>(1)</sup> got similar results in two varieties and eleven alfalfa lines, with an average 20,615 kg DM ha<sup>-1</sup>. Abusuwar and Daur<sup>(20)</sup> found, in Cuf 101 and Hegazi varieties, higher and lower yields of 18,065 and 17,545 kg DM ha<sup>-1</sup>. The greatest total accumulated alfalfa forage production was reported in the Valley of Mexico, with 33,864 and 34,457 kg DM ha<sup>-1</sup>, respectively; the greatest seasonal distribution was in spring and summer, and the lowest in fall and winter, with the same cutting intervals as in this investigation<sup>(5,8)</sup>. Nevertheless, lower yields were observed in this study and could be attributed to the fact that the varieties had been established for more than 2 years (April 2008), so that forage persistence and yield decreased over time, after being sown<sup>(4)</sup>.

The analysis of variance did not reveal interactions (P>0.05) between the studied factors. Differences were found (P<0.05) in annual average weight per stem, between the alfalfa varieties: Aragon, Milenia and Chipilo produced heavier stems (0.71 g, average) than Valenciana and Oaxaca, with 0.67 y 0.68 g, respectively (Table 2). A seasonal effect was found in all varieties (P<0.05), the average weight per stem was greater in spring and lower in winter, with respect to the rest of the seasons. The highest values were observed in spring and were associated with the maximum temperatures recorded during the study (Figure 1). Nevetheless, the authors mention that these differences can also be due to cutting frequencies<sup>(3)</sup>; upon evaluation of the alfalfa cutting frequency, these authors found the lowest and highest weight per stem to be 0.27 and 0.45 g, for the lowest and highest frequency, respectively.

Variety	Summer	Fall	Winter	Spring	SEM	Average
Aragon	0.83 Aa	0.69 Aab	0.36 Bb	0.94 Aa	0.11	0.71 A
Valenciana	0.80 Aa	0.69 Ab	0.33 Bc	0.86 Ba	0.16	0.67 B
Chipilo	0.72 Ba	0.70 Aab	0.45 Ab	0.93 Aa	0.12	0.70 A
Milenia	0.75 Bb	0.71 Ab	0.34 Bc	1.04 Aa	0.21	0.71 A
Oaxaca	0.74 Ba	0.67 Aab	0.45 Ab	0.86 Ba	0.15	0.68 B
SEM	0.9	0.7	0.6	0.9		0.8
Average	0.75 b	0.69 b	0.39 c	0.93 a		

**Table 2:** Seasonal changes in weight per stem (g) for alfalfa varieties

abc= means with the same lowercase letter in the same row are not different (P>0.05). ABC= means with the same uppercase letter in the same column, are not different (P>0.05). SEM= Standard error of the mean.

Meuriot *et al*<sup>(21)</sup> evaluated the alfalfa cutting frequency and intensity and found that stem weight was larger (1.1 g per stem) as cutting frequency increased, with a 15 cm cutting intensity; this was related to a greater leaf area index (LAI) and yield. Avci *et al*<sup>(13)</sup> reported that the greater weight per stem was associated with a better yield, as was seen in the spring, during this investigation. The increase in weight per stem coincides with the decrease in stem density, mainly in spring. This behavior has been reported by other authors<sup>(22)</sup>, who point out that the increase in stem density per unit of area causes a decrease in individual stem weight; this is explained by the self thinning law<sup>(23)</sup> and confirmed by other authors<sup>(24,25,26).</sup>

The interactions between alfalfa varieties and the times of the year were not significant (P>0.05) with respect to this response variable. There were differences (P<0.05) between the varieties, since the Aragon had the greatest average annual stem density, with 634 stems m<sup>-2</sup>, while the Oaxaca variety had the lowest density, with 512 stems m<sup>-2</sup> (Table 3). Seasonal differences (P<0.05) also existed, since the average stem density in summer was greater than the one recorded in winter; however, the lowest stem density was recorded in spring.

Variety	Summer	Fall	Winter	Spring	SEM	Average
Aragón	715 Aa	660 Aab	585 Ab	577 Ab	234	634 A
Valenciana	708 ABa	684 Ab	483 Bd	503 Bc	145	595 B
Chipilo	739 Aa	692 Aa	525 ABb	318 Cc	124	568 B
Milenia	666 BCa	592 BCb	528 ABbc	496 Bc	98	571 B
Oaxaca	623 Ca	537 Cb	518 ABb	372 Cc	134	512 C
SEM	97	78	102	87		65
Average	690 a	633 ab	528 b	453 c		

**Table 3:** Seasonal changes in stem density (stems m<sup>-2</sup>) of alfalfa varieties

<sup>abc</sup>= Means with the same lowercase letter in the same row are not different (P>0.05). ABC= means with the same uppercase letter in the same column, are not different (P>0.05). SEM= Standard error of the mean.

In another investigation<sup>(27)</sup> of four alfalfa varieties, the authors observed the same behavior as in this study, since stem density decreased as the study progressed. They recorded greater stem density in the first year of evaluation and the lowest in the fourth year, with an average 518 and 140 m<sup>-2</sup>, respectively. Nevertheless, Chen *et al*<sup>(28)</sup> stated that as the cutting frequency decreased, the stem density increased until it reached a point of decline, independently of the variety and year of evaluation: 645, 734 and 688 stems m<sup>-2</sup> for cutting frequencies every 30, 40 and 50 d, respectively, which is highly related to yield.

Soil temperature and humidity are the main climatic factors having an influence on stem density and weight; when these are favorable, there is constant stem production, resulting in a greater biomass production in the prairie<sup>(29)</sup>. However, an inverse relationship has been mentioned<sup>(22)</sup> between stem density and dry matter production. Researchers point out that a greater number of stems results in a lower forage yield, possibly due to the low individual weight.

The analysis of variance did not show significant interactions (P>0.05) between the factors under study. Just as with stem density, average plant density decreased (P<0.05) in all alfalfa varieties, as the study progressed (Table 4), from 33 plants m<sup>-2</sup> in summer, to 22 plants m<sup>-2</sup> in spring. The greatest average annual plant density was recorded for Milenia, with 33, and the lowest for Aragon, with 21 plants m<sup>-2</sup>. Both lost 9 and 11 plants between the beginning and end of the study, respectively. Other authors<sup>(30)</sup> mention that, in an alfalfa prairie, plant cover and density are stabilized, as time since its establishment increases; however, a time comes when these decrease, depending on the variety and the site.

Variety	Summer	Fall	Winter	Spring	SEM	Average
Aragon	26 Ca	22 Db	20 Cb	17 Cc	3	21 C
Valenciana	34 Ba	32 ABb	26 Bc	22 Bd	2	29 B
Chipilo	33 Ba	29 BCab	26 Bbc	23 Bc	3	28 B
Milenia	38 Aa	36 Ab	31 Ac	27 Ad	2	33 A
Oaxaca	31 Ba	27 Cb	25 Bbc	22 Bc	3	26 B
SEM	3	4	5	4		3
Average	33 a	29 b	26 c	22 d		

**Table 4:** Seasonal changes in plant density (plants m<sup>-2</sup>) for alfalfa varieties

<sup>abc</sup>= Means with the same lowercase letter in the same row are not different (P>0.05). ABC= means with the same uppercase letter in the same column, are not different (P>0.05). SEM= Standard error of the mean.

Another study<sup>(31)</sup> mentions the importance of distance between the alfalfa plants. They found the highest yield in spring, related to the greatest intercepted radiation (95%) in all distances between plants (10, 15, 20, 25 and 30 cm); while in summer and winter, 95% intercepted radiation was only reached at a distance of 10 and 15 cm between plants, since alfalfa growth is related to temperature. Several authors<sup>(27,32)</sup> state that the smaller the separation between plants, the higher is the yield, which coincides with this study's findings.

No interaction was recorded between the alfalfa varieties and the seasons, for this response variable. However, the leaf:stem ratios varied (P<0.05) in the different seasons (Table 5): in fall and winter there was higher average leaf:stem ratio (1.52) which was significantly different from the summer and spring relationship (0.92). On the other hand, the Aragon and Valenciana varieties showed the highest leaf:stem ratio (1.30), compared to the Chipilo and Oaxaca varieties (1.14). In a study by Rojas *et al*<sup>(33)</sup>, they observed that independently of the variety, in fall and winter the leaf:stem ratio was greater, with a value of 1.49, compared to the value recorded in summer and spring, with 0.92 and 0.94 respectively, Villegas *et al*<sup>(34)</sup> observed that, with two cutting intensities, the Moapa and Tlacolula varieties had the best and worst leaf:stem ratio with 1.4 and 1.1 respectively. Other authors<sup>(8)</sup> reported values much lower than the above, and than the ones of the present study, since the annual average observed in five alfalfa varieties was 0.79, with variations throughout the year, and highest and lowest values (P<0.05) were observed in January and November, with 1.05 and 0.62, respectively. Also, Morales *et al*<sup>(15)</sup> recorded an average annual leaf:stem ratio of 0.68, in fourteen alfalfa varieties.

Variety	Summer	Fall	Winter	Spring	SEM	Average
Aragon	0.94 Ab	1.65 Aa	1.66 Aa	0.99 Ab	0.23	1.31 A
Valenciana	0.92 Ab	1.59 Aa	1.69 Aa	0.96 Ab	0.32	1.29 A
Chipilo	0.84 Bb	1.40 ABa	1.44 Ba	0.94 Ab	0.21	1.15 B
Milenia	0.95 Ab	1.49 ABa	1.50 ABa	0.97 Ab	0.19	1.23 AB
Oaxaca	0.87 Bb	1.38 Ba	1.44 Ba	0.83 Bb	0.18	1.13 B
SEM	0.7	0.15	0.15	0.15		0.13
Average	0.90 b	1.50 a	1.55 a	0.94 b		

**Table 5:** Seasonal changes in leaf:stem ratio in five alfalfa varieties

<sup>abc</sup>= Means with the same lowercase letter in the same row are not different (P>0.05). ABC= means with the same uppercase letter in the same column, are not different (P>0.05). SEM= Standard error of the mean.

Hernández-Garay *et al*<sup>(23)</sup> mention that the leaf:stem ratio of forage may be considered an indirect measurement of quality, since values greater than one show a better forage quality, having a greater amount of leaves. In this study, indexes greater than 1 were recorded in fall and winter. However, even though in these seasons alfalfa plants produced a higher leaf:stem ratio, dry matter yield tended to be lower in fall, and was the lowest in winter, compared to spring and summer (Table 1). Rojas *et al*<sup>(4)</sup> mention that in forage, one must obtain the best relationship between yield and quality, which happens when there is a greater amount of leaves.

Independently of the variety, alfalfa constituted more than 90% of the desirable species in the prairie, during the whole study period (Figure 2). Differences were observed between seasons in the percentage of leaves, with a greater leaf contribution, 59%, in fall and winter, and a lower one, 45%, in spring and summer. With respect to stem percentage, the greatest contribution was found in spring and summer, and the lowest in fall and winter. No dead matter was found during the whole experimental period, since alfalfa tends to shed its senescent leaves. Inflorescences were also not found, due to the fact that the cuttings were done before the inflorescences appeared. Several researchers<sup>(5,6,8)</sup> reported similar behavior with respect to the amount of alfalfa leaves, which were most abundant during periods with the lowest temperatures.





Only in summer was there a greater presence of weeds, compared to other seasons, being the main ones: *Aristida stricta, Bromus inermis* and *Malva neglecta*. The higher percentage of weeds in summer could be due to higher temperatures and rainfall recorded in this season (Figure 1), which are appropriate for weeds, with intraspecific competition existing with alfalfa, for light, water and nutrients<sup>(30)</sup>. The varieties that showed the greatest weed invasion were Valenciana and Oaxaca, with 9 %. The greater presence of weeds in these varieties could be attributed to lower plant density (Table 4), which led to a greater invasion by undesired weeds, as reported in other works<sup>(5,14)</sup>.

It was conclude that alfalfa varieties displayed different behaviors and that the highest yield was found in Milenia, Chipilo, Oaxaca and Valenciana. According to the statistical differences between the general averages of each season, the dry matter yield in summer was mostly contributed by stem density and plants; also, a greater stem weight led to higher dry matter yield in spring. However, it is necessary to continue carrying out investigations and include other production

parameters, such as plant height, intercepted radiation and leaf area index, which could better explain alfalfa's productive behavior in each season; this could contribute to improvement in crop management.

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