

GROWTH ANALYSIS OF WHITE CLOVER (*Trifolium repens* L.) AND INDIRECT METHODS TO ESTIMATE ITS FORAGE YIELD

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ABSTRACT

Dry matter (DM) accumulation and its relationship to some indicators of physiological efficiency on white clover (*Trifolium repens* L.) growth was studied to determine the optimal harvesting time by season. The experiment was carried out at the Colegio the Postgraduados *Campus* Montecillo, Texcoco, Mexico. Twenty-four plots, each measuring 3.7 X 1.7 m, were used, and distributed in a completely randomized design with eight treatments and three replicates. The treatments consisted of weekly successive cuts during an eight-week sprouting cycle during the middle of each season of the year. The evaluated variables were dry matter accumulation, crop growth rate (CGR), intercepted radiation (IR), and plant height (cm) estimated through the ruler and rising plate meter methods. Most herbage accumulation ($p \leq 0.05$) occurred at the eighth week of the spring (2,688 kg DM ha⁻¹). Most CGR ($p \leq 0.05$) occurred at the first week of the summer (84 kg DM ha⁻¹ day⁻¹). The largest IR occurred at the sixth and ninth week of the summer (100 %). Maximum height occurred at week eight of the summer ($p \leq 0.05$), which was 32 and 23.3 cm for the ruler and the rising plate meter methods, respectively. Dry matter yield increased linearly due to the favorable climatic conditions during spring-summer, where there was a higher intercepted radiation and higher crop growth. The ruler method showed a higher coefficient compared to the ascending plate, so the ruler method for white clover can be recommended.

Keywords: legume, dry matter accumulation, intercepted radiation.

INTRODUCTION

Due to the effects of climate change on agriculture and the higher animal nutritional requirements, a consequence of the genetic improvement, it is necessary to have forages that are well adapted to various climatic conditions, that persist under grazing or cut, and that show good biomass yield and nutritional quality (Rojas *et al.*, 2016a).

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The adaptation of the “rational” grazing system is in its heyday in Mexico and Latin America. This system incorporates short grazing intervals and a high animal load, which allows a rapid recovery of plant communities (Conant *et al.*, 2003; Teague and Barnes, 2017); the main benefits include a reduction of excessive grazing and soil erosion, improved herbage use, animal productivity, and higher carbon (C) sequestration from the soil (Teague *et al.*, 2016).

White clover (*Trifolium repens* L.) is considered as the most common and agriculturally important legume in grazing systems in all the temperate regions of the world. Its high biomass yield and herbage quality, as well as nitrogen (N) contribution and its stoloniferous growing habit, which minimizes losses of growing points, make white clover a valuable forage crop (Black *et al.*, 2009). Potential N fixation rates are in the range of 600 to 700 kg N ha⁻¹ yr⁻¹ (Luscher *et al.*, 2001); it has high protein content, low structural fiber and high organic matter digestibility. However, its performance is better in low fertility soils (Conant *et al.*, 2003). The intensive temperate milk production systems in Mexico animal production is based on confined animals, and it depends on grain and cereal imports, which travel long distances to reach their destination, thus disrupting the natural cycles of carbon and nutrients (Steinfeld *et al.*, 2006).

The quality of the dry matter depends on the intercepted radiation, age, forage growth rate, season of the year (Rojas *et al.*, 2016b). Grazing must be managed according to the meadow, for this, rapid and practical techniques must be available to estimate forage availability and subsequent recovery and obtain the optimal grazing moment (Castro *et al.*, 2012). There are different methods to measure the dry matter of forages, the most common is the fixed square, which is the direct method to collect fresh forage that is taken to the laboratory for drying in forced air ovens. Research on white clover has been conducted at the Central Mexican Plateau and the crop has shown good performance under grazing conditions in pure swards, where its maximum yield is reached during spring, autumn and winter at the eighth week of regrowth with 2953, 1592, 1791 kg DM ha⁻¹, respectively (Gutiérrez *et al.*, 2018). However, there are also indirect methods, such as the height of the rising plate and the ruler, which allow rapid non-destructive measurements to be made (Rojas *et al.*, 2021). Therefore, the objective of this study was to evaluate dry mass accumulation, crop growth rate, intercepted radiation and to define the optimal height method with ruler and rising plate in white clover.

MATERIALS AND METHODS

The assay was carried out in a white clover variety Ladino sward at the experimental field of the Colegio de Postgraduados, at Montecillo, Texcoco, State of Mexico, at 19° 29' N and 98° 53' W, at an altitude of 2,240 m. Broadcast seeding was carried out in February using pure viable seeds at of a 6 kg ha⁻¹ rate. The climate of the location is temperate subhumid, with a mean annual precipitation of 636.5 mm and a summer rainy season (June to October) and mean annual temperature of 15.2° C (García, 2004). The soil was analyzed at the Plant Nutrition Laboratory S.A. and it was identified as a sandy loam soil, with pH 8.4 and 3.5 % of organic matter.

Before starting the study and at the middle of each season a homogenization grazing was carried out and afterwards, an eight-week growth analysis was performed on a weekly basis. Sheep were used for grazing until a remnant of 5 cm above the soil level was left, and to improve management, an electric fence was set up around the experimental plots. Twenty-four plots were drawn, each of 3.7 x 1.7 m, where the eight treatments three replicates were randomly assigned by the irregular slope of the terrain. The treatments included forage production evaluations at week one, week two, week three, and so on, up to cuts at week eight of regrowth age following the recommendations of researchers in growth analysis (Lane *et al.*, 2000). It should be noted that each time a growth analysis per season was completed, the meadows were left to rest until another season arrived to start with the homogenization grazing and perform another growth analysis. During periods of low soil water levels, plots were flood irrigated every two weeks and no fertilization was applied.

Monthly outdoor temperature averages (maximum and minimum) and monthly precipitation during the period of study were obtained from the agrometeorological station of Colegio de Postgraduados, located 100 m from the experimental site. The maximum monthly temperature varied from 22.1 to 30.2 °C, whereas the minimum temperature varied from 0 to 11 °C (Figure 1). The highest temperature occurred during spring, in May, which was 30.2 °C; the lowest temperature was registered during winter with 0 °C in December. Accumulated precipitation from March to April was 613 mm, of which 75.8 % occurred during June, July, August, September, and October, with an accumulated precipitation of 465 mm.

After the homogenization grazing, three 0.25 m² squares were cut at a height of 5 cm from the ground in each experimental plot during eight weeks. The herbage harvested

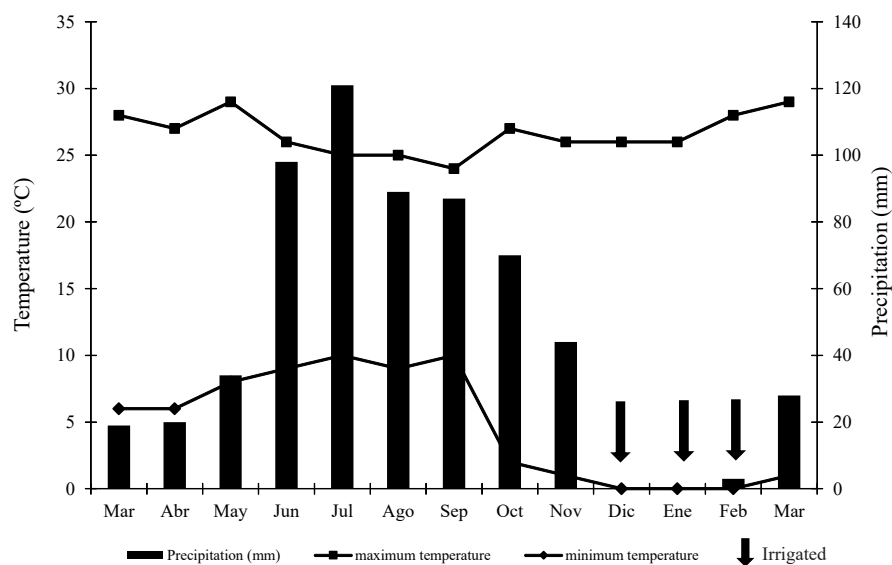


Figure 1. Maximum and minimum mean monthly temperatures and accumulated precipitation during the period of study at the Central Mexican Plateau in Montecillo, Texcoco, State of Mexico.

from each square was rinsed and subsequently dried in labeled paper bags inside a forced air oven to 55 °C during 72 hours in order to estimate the amount of dry matter per hectare at the different regrowth ages.

Growth rate was calculated from the yield data obtained at each cut in each replicate through the following formula:

$$GR = Y / T$$

Where:

GR= Mean seasonal growth rate (kg DM ha⁻¹ d⁻¹).

Y= Seasonal herbage yield (kg DM ha⁻¹).

T= Days elapsed after the last cut.

Intercepted radiation was calculated before each grazing event through the wooden ruler method. For that, five readings were taken in each experimental unit and it consisted in sliding a 1 m wooden ruler under the plant canopy at a north-south orientation. Afterwards, millimeters that were in the shade were counted summarized, which represented the percentage of intercepted radiation by the plant canopy. The reading was carried out between 12:00 and 13:00 h of the corresponding time zone, since it is the optimal moment due to the sun's angle and because the light intercepted changes minimally (Rojas *et al.*, 2016b). The indirect methods used to estimate the amount of herbage at a given moment relate canopy height to dry matter yield through linear regression. Plant height was measured before and after a cut, and 20 samples were randomly taken from the whole experimental unit using a ruler and a rising plate.

The ruler method consisted in placing a 100 cm graduated ruler with a 1 mm accuracy above the plant canopy and sliding it until the first morphological component and the data was recorded. The rising plate method consisted in a 900 cm² aluminum plate that moved freely upwards or downwards on a central column. To estimate height with this instrument, it was placed vertically above the forage and, once it was supported by the forage mass, the data was recorded (López *et al.*, 2011).

To calibrate both sampling techniques, before cutting the forage present in the four assigned squares, readings were taken with the ruler and the rising plate. Afterwards, a linear regression equation was obtained, which correlated the data gathered through sampling by each technique with the dry matter yield accumulated in each square. After the equation was determined, the average values per cut from each treatment were replaced in order to obtain yield values of dry matter by cutting. Data was analyzed using the GLM procedure of SAS (SAS, 2009) for a completely randomized experimental design, where the treatments were the eight weeks evaluated in the middle of each season with three replicates and a regression analysis for each variable.

RESULTS AND DISCUSSION

The results of dry matter (DM) yield are shown in Table 1. The yield of DM increased with the age, and reached the maximum at the eighth week in spring and autumn with

Table 1. Weekly changes of dry matter accumulation (kg DM ha⁻¹) white clover (*Trifolium repens* L.) at the Central Mexican Plateau in Montecillo, Texcoco, State of Mexico.

Week	Spring	Summer	Autumn	Winter
1	136± 2.3 Dd	586±10.3 Ca	187± 3.3 Dc	456± 9.0 Cb
2	324± 9.5 CDc	702±13.2 Ca	220± 4.4 Dd	464±10.1 Cb
3	720±12.6 BCb	1066±23.1 BCa	595±10.5 CDc	667±12.4 BCb
4	1029±22.9 Bb	1268±28.2 ABCa	662±11.2 CDd	839±14.2 BCc
5	1084±24.2 Bc	1649±42.2 ABCa	725±14.2 BCDd	1399±22.3 ABb
6	1253±27.2 Bc	1982±39.5 ABa	1153±25.6 ABCc	1642±33.2 Ab
7	2171±40.2 Aa	2242±43.2 Aa	1759±44.2 Ab	455± 9.2 Cc
8	2688±55.3 Aa	1842±32.4 ABb	1781±41.3 Ab	1274±21.4 ABC
Mean	1175±33.6 b	1403±32.1 a	954±29.4 c	885±26.9 d
SEM	30	20	21	15
Significance	**	*	**	*

ABCD= Different capital letters within each column show significant differences; abcd= Different lowercase letters within rows show significant differences. SEM = standard error of the mean; Significance; * = $p \leq 0.05$; ** = $p \leq 0.01$.

2688 and 1781 kg DM ha⁻¹, respectively ($p \leq 0.05$); at the seventh week in the summer (2241 kg DM ha⁻¹); and at the sixth week in winter (1643 kg DM ha⁻¹). Accumulated biomass during spring was higher by 20 % (447 kg DM ha⁻¹), 51 % (907 kg DM ha⁻¹) and 64 % (1,045 kg DM ha⁻¹), compared with summer, autumn and winter, respectively.

When evaluating a monoculture of white clover Gutiérrez *et al.* (2018) at the Central Mexican Plateau, found that dry matter yield increased as the age of the regrowth increased in all the seasons, and it reached the maximum yield at the eighth week or regrowth age for spring, autumn and winter, with 2953, 1592, 1791 kg DM ha⁻¹, respectively, and at the seventh week of winter with 1971 kg DM ha⁻¹. Moreno *et al.* (2015) when established white clover with mixtures of ryegrass *Lolium perenne* (L.) and orchardgrass *Dactylis glomerata* (L.) found a maximum yield of white clover of 513 kg DM ha⁻¹ during the first sampled year, much lower than in this study. However, Maldonado *et al.* (2017) with these same mixtures but at the third and fourth year of production, recorded a significant increase in white clover yield, with an average of white clover of 7220 kg DM ha⁻¹. On the other hand, Rojas *et al.* (2016a) obtained the best grass-legume mixture with 40 % of white clover, 40 % of orchardgrass and 20 % ryegrass, which gave an accumulated annual yield of 20182 kg DM ha⁻¹, whereas during spring, the highest yield was obtained, with 7292 kg DM ha⁻¹, followed by summer with 5923 kg DM ha⁻¹.

White clover growth rate varied in the different seasons of the year. The highest growth rate occurred at the eighth week in spring (48 kg DM ha⁻¹ day⁻¹) and at the sixth week for summer (44 kg DM ha⁻¹ day⁻¹). However, the seasons with the lowest growth rate were those with the lowest temperatures (Figure 1), autumn and winter, where the highest growth was obtained at the fifth and eighth week, with 27 and 29 kg DM ha⁻¹ day⁻¹, respectively (Figure 2).

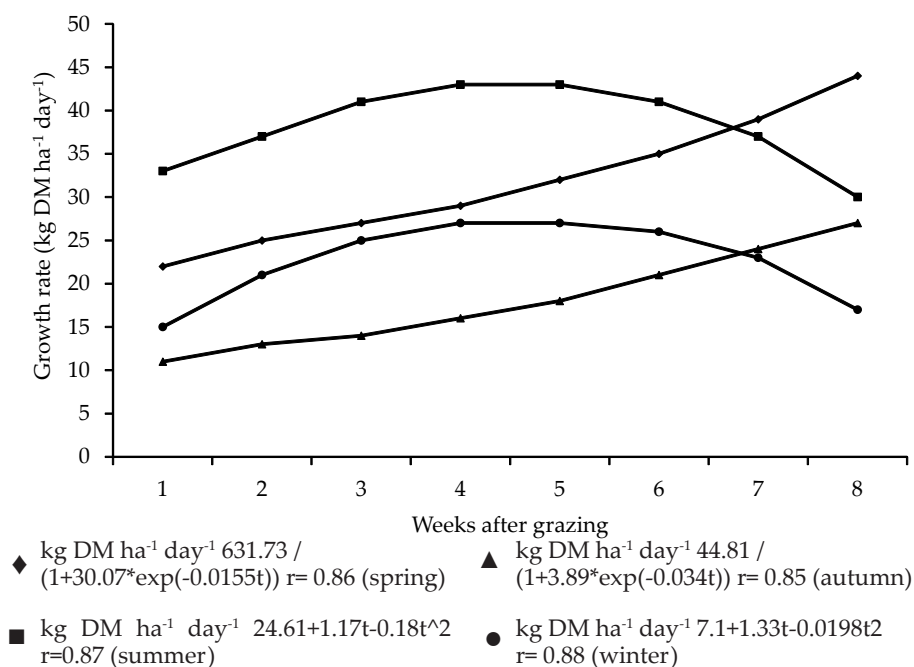


Figure 2. Weekly changes of white clover (*Trifolium repens* L.) growth rate after cutting at the Central Mexican Plateau in Montecillo, Texcoco, State of Mexico.

In another study on white clover, the highest growth rate was obtained during spring, with 48 kg DM ha⁻¹ day⁻¹ at week eight, followed by summer, winter and autumn, with 33, 29 y 28 kg DM ha⁻¹ day⁻¹, for the weeks five, eight and five, respectively (Gutiérrez *et al.*, 2018), something similar to what was found in this study. In some studies, carried out in Mexico with mixed swards with white clover Castro *et al.* (2012) reported the highest seasonal growth rate during summer in week 4 (64 kg DM ha⁻¹ day⁻¹), higher by 21, 55 and 186 % than the growth rates in spring in week 4 (50 kg DM ha⁻¹ day⁻¹), winter in week 6 (41 kg DM ha⁻¹ day⁻¹) and autumn in week 5 (20 kg DM ha⁻¹ day⁻¹). On the other hand, Rojas *et al.* (2016a) in associations with white clover, reported higher growth rates during spring (81 kg DM ha⁻¹ day⁻¹), whereas for summer it was 56 kg DM ha⁻¹ day⁻¹, and in autumn and winter, the rates were lower, with 35 and 29 kg DM ha⁻¹ day⁻¹, respectively.

The seasonal growth rate of white clover matches the results obtained at other latitudes of temperate climate, where the highest growth rate has occurred during spring-summer, since the elevated light levels in this period allow the plant to have higher tissue replacement rates and leaf generation and expansion (Black *et al.*, 2006; Black *et al.*, 2009) due to precipitation and temperatures favorable to the plant of 18–30 °C (Black *et al.*, 2006) with an optimum of 25 °C (Lane *et al.*, 2000). On the other hand, low temperatures and limited radiation only increase petiole lengthening, inhibit branching and stolon size (Black *et al.*, 2006).

Most of the radiation was intercepted during summer, at the sixth week of sprouting (100 %), which was superior to weeks one and two. In spring (98.6 %) and autumn (99.6 %), the maximum radiation was intercepted at week eight, whereas during winter it occurred at week five (95 %), which was similar to week six but different from the remaining weeks of the sampling (Figure 3).

In a study carried out in mixed swards Flores *et al.* (2015) reported that there was more intercepted radiation during spring (92 %) and summer (93 %), whereas autumn and winter obtained the lowest values of 88 and 86 %, respectively. White clover leaves have high photosynthetic capacity when in monoculture; however, when it is associated with C₃ grasses such as ryegrass and orchardgrass, competition for light, space, water and nutrients limit white clover performance (Herault-Bron *et al.*, 1999; Maldonado *et al.*, 2017), moreover, C₃ grasses are more photosynthetically efficient than legumes (Faurie *et al.*, 1996).

The wide distribution of the genus *Trifolium* (approximately 300 species) tend to behave differently depending on the latitude due to their morphological pattern

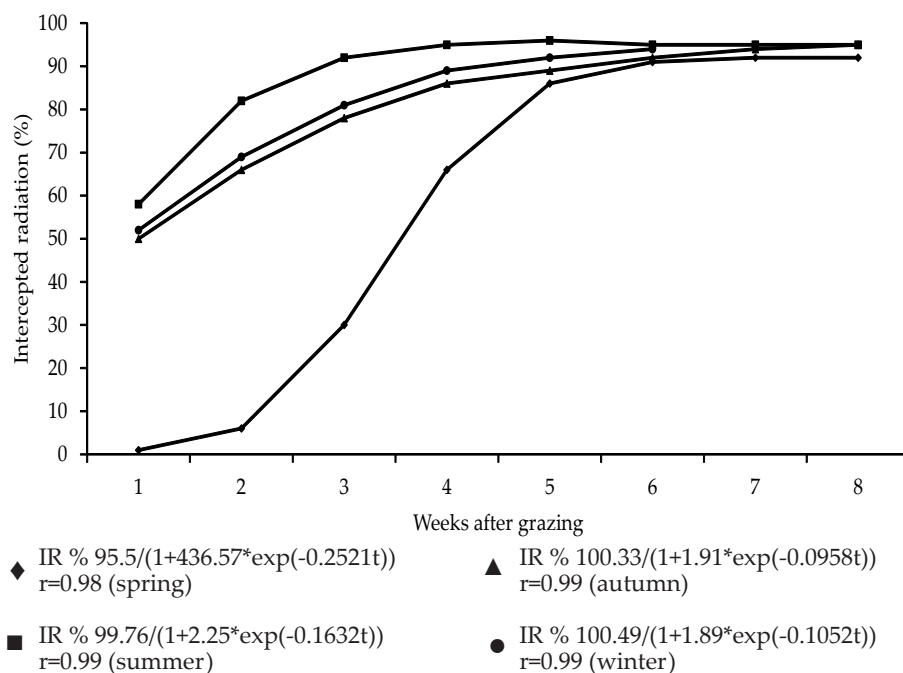


Figure 3. Weekly changes of intercepted radiation during seasonal growth of white (*Trifolium repens* L.) at the Central Mexican Plateau in Montecillo, Texcoco, State of Mexico. Photosynthetically active radiation (PAR) from sunlight is absorbed or reflected by the first surface it touches, thus, if it is not intercepted by green leaves, its energy will not be available for plant growth. The capacity of the white clover and associated grasses to develop a canopy of healthy green leaves is important to maximize yield and ensure sward persistence; therefore, the development of this canopy depends on the components of the expansion of leaf area, senescence and canopy architecture (Black *et al.*, 2009).

(Randazzo *et al.*, 2013). For example, southern latitude populations develop thicker with long petioles and wide leaves, which allows them to collect more light; however, northern latitude populations develop thinner stolons, and thinner, prostrate leaves, which limits their growth and development (Luscher *et al.*, 2001).

Plant height increased with the weeks of regrowth (Table 2). Both methods recorded the maximum height at week eight during spring-summer and autumn ($p \leq 0.05$); however, during winter, the maximum height was 17 cm, recorded at the sixth week by the ruler method and 15 cm by the rising plate method.

During an evaluation of sward height with the ruler method, Castro *et al.* (2012) found greater heights during summer (26 cm), which were superior by 17, 142 and 137 % than the heights of spring, autumn and winter, respectively. According to other authors (López *et al.*, 2011; Rojas *et al.*, 2016a), height is a useful tool to estimate herbage yield present at a given moment. According to other researchers (Dillard *et al.*, 2016), herbage mass present in the sward is easy to measure and estimate through simple sampling, taking as a reference the average of 15 to 45 cm height, which allows to decide when to let the cattle in or out in order to use the sward efficiently.

With the height information obtained (Table 3) and dry matter yield accumulated in each square (0.25 m²), linear regression equations and coefficients of determination (r^2)

Table 2. Weekly changes in sward height (cm) s of white clover (*Trifolium repens* L.) at Central Mexican Plateau in Montecillo, Texcoco, State of Mexico.

Week	Spring	Summer Ruler method (cm)	Autumn	Winter
1	2.7±0.2 Cc	12.3±1.0 Ea	6.7±0.4 Bb	6.3±0.2 Db
2	6.8±0.5 Cb	15.7±1.2 EDa	7.3±0.7 Bb	6.7±0.3 Db
3	7.0±0.6 Cb	20.7±1.2 CDa	7.7±0.6 Bb	9.3±0.5 Cb
4	7.0±0.6 Cb	21.0±1.4 Ca	9.7±0.7 ABb	11.0±0.6 BCb
5	12.7±1.2 Bb	23.7±1.7 BCa	13.0±0.9 Ab	12.7±0.8 Bb
6	15.5±1.4 Bbc	23.3±1.6 BCa	13.3±1.0 Ac	17.0±1.1 Ab
7	18.0±1.6 ABb	27.3±1.7 Aba	13.3±1.0 Ab	—
8	23.3±2.1 Ab	32.0±1.8 Aa	14.0±1.1 Ac	—
SEM	1.1	1.4	0.9	0.5
Significance	**	**	**	**
		Rising plate method (cm)		
1	3.3±0.3 Eb	10.0±0.7 Ca	6.0±0.4 CDb	5.7±0.3 Bb
2	3.7±0.4 Eb	15.7±0.9 BCa	5.3±0.3 Db	6.0±0.4 Bb
3	3.7±0.4 Eb	23.0±1.3 Aa	7.0±0.5 CDb	6.3±0.5 Bb
4	7.3±0.5 DEb	23.7±1.4 Aa	11.3±0.9 ABCDb	9.0±0.8 Bb
5	8.7±0.6 CDb	23.7±1.6 Aa	7.7±0.6 CDb	10.7±0.9 ABb
6	12.3±0.9 BCb	26.3±1.8 Aa	13.0±1.0 ABCb	15.0±1.3 Ab
7	16.7±1.1 Bb	21.3±1.6 ABa	14.3±1.1 ABc	—
8	22.7 Aa	23.3±1.9 Aa	17.3±1.3 Ab	—
SEM	0.9	1.4	0.8	0.7
Significance	**	**	**	*

ABCD= Different capital letters within column show significant differences; abcd= Different lowercase letters within rows show significant differences. SEM = standard error of the mean; Significance; * = $p \leq 0.05$; ** = $p \leq 0.01$.

Table 3. Linear regression equations obtained by season and whole year based on sward height and related to dry matter yield of white (*Trifolium repens* L.) at the Central Mexican Plateau in Montecillo, Texcoco, State of Mexico.

Method		Spring	Summer	Autumn	Winter	Annual
Ruler	Equation	$y = 113.74x - 46.71$	$y = 78.1x - 464.88$	$y = 135.26x - 727.86$	$y = 111.52x - 459.34$	$y = 71.98x - 21.60$
	r^2	0.85	0.54	0.64	0.81	0.56
Plate	Equation	$y = 113.71x + 62.1$	$y = 70.84x - 226.27$	$y = 102.75x - 343.98$	$y = 114.64x - 294.66$	$y = 68.6x + 110$
	r^2	0.84	0.36	0.74	0.83	0.52

were obtained for each season and year. To use the indirect methods, it is necessary to measure plant height (cm) and dry matter present in the square (0.25 m²), since both variables have a highly positive correlation (López *et al.*, 2011).

For spring, the data had a better fit to the model for the ruler ($r^2 = 85$) than the rising plate method ($r^2 = 84$). Likewise, in the annual regression equation, the ruler method showed higher coefficient ($r^2 = 0.56$) than the rising plate method ($r^2 = 0.52$); which indicates that the variation of dry matter yield per hectare is explained by 56 % by the height with the ruler and 52 % with the ascending plate, obtaining 111 and 87 kg DM ha⁻¹ for each cm of height of the estimated plant with the ruler and rising plate, respectively. Based on the results obtained and the goodness of fit of the model, the ruler method could be more reliable (Bransby *et al.*, 1977); however, different studies have reported variability in model fitness for both methods; for example, in another investigation Ganguli *et al.* (2000), reported a higher correlation coefficient with the rising plate method ($r^2 = 0.83$) and ($r^2 = 0.60$) through the ruler method. On the other hand, Castillo *et al.* (2009) found a better correlation through the ruler method than through the rising plate method, which showed a r^2 higher than 0.83.

Determination coefficients in linear regressions by both methods are reliable and acceptable to estimate dry matter present at a given moment, using sward height (cm). However, although several authors (López *et al.*, 2011) debate the accuracy and precision of both indirect methods, all of them agree that these methods are useful and easy to implement for sward management, since they help to make a good decision to use forage efficiently.

CONCLUSIONS

Dry matter yield increased linearly due to the favorable climatic conditions during spring-summer, where there was a higher intercepted radiation and higher crop growth than the other seasons. Similarly, the indirect methods showed a larger height for the spring-summer period, which coincided with the accumulated dry matter; however, even though the indirect methods are not exact, it is recommended to calibrate them with fixed squares before each sampling. The ruler method showed

a higher coefficient compared to the ascending plate, so the ruler method for white clover can be recommended.

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