



Cut management of *Tithonia diversifolia* as a function of regrowth ages: a physiological and morphological approach

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[*Manejo de corte de Tithonia diversifolia em função da idade de rebrota: uma abordagem fisiológica e morfológica*]

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ABSTRACT

Tithonia diversifolia is exploited in tropical countries as a forage. However, there are no studies on its ecophysiology and relationship with the management of the species. This study aimed to evaluate the post-cut regrowth ages of *T. diversifolia* in terms of physiological and productive behavior and its implications for forage management. A completely randomized design was used, with four replications and ten regrowth ages of *T. diversifolia* at 21, 28, 35, 42, 49, 56, 63, 70, 77, and 84 days after the cut. Photosynthetic rate and stomatal conductance showed relatively low values at the beginning of growth and maximum values at 39 and 51 days of regrowth and subsequent decline. WUE was higher at 45 days of regrowth. A linear reduction in the function of the plant's age was observed for transpiration. Leaf dry mass reflected the balance of physiological processes, with a gradual increase and subsequent decrease in net production. A period of 15 days, between 49 to 63 days of regrowth, is recommended for using *T. diversifolia* as forage. This period associates leaf availability, higher carbon fixation, more significant WUE, and little senescent material (<5% of the plant's total dry mass) from *T. diversifolia*.

Keywords: adaptive capacity, ecophysiology, efficiency of water use, mexican sunflower, photosynthetic capacity

RESUMO

Tithonia diversifolia é explorada em países tropicais como planta forrageira. Porém, não existem estudos sobre sua ecofisiologia e a relação com o manejo da espécie. Objetivou-se avaliar as idades de rebrota pós-corte de *T. diversifolia* em termos de comportamento fisiológico e produtivo e suas implicações no manejo da forrageira. Foi utilizado delineamento inteiramente ao acaso, com quatro repetições e 10 idades de rebrota de *T. diversifolia* aos 21, 28, 35, 42, 49, 56, 63, 70, 77 e 84 dias após o corte. A taxa fotossintética e a condutância estomática apresentaram valores relativamente baixos, sendo observados valores máximos aos 39 e 51 dias de rebrota e posterior declínio. A eficiência do uso da água foi maior aos 45 dias de rebrota. Para a transpiração, foi observada redução linear em função do aumento da idade da planta. A massa seca foliar refletiu o equilíbrio dos processos fisiológicos, com aumento gradativo e posterior diminuição da produção líquida. Recomenda-se um período de 15 dias, entre 49 e 63 dias de rebrota, para utilização de *T. diversifolia* como forragem. Esse período associa disponibilidade foliar, maior fixação de carbono, eficiência do uso da água mais significativa e baixa porcentagem de material senescente (<5% da massa seca total da planta) de *T. diversifolia*.

Palavras-chave: capacidade adaptativa, ecofisiologia, eficiência no uso da água, girassol mexicano, capacidade fotossintética

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INTRODUCTION

Tithonia diversifolia (Hemsl.). A. Gray is commonly known as Mexican sunflower, golden button, "soil-arnica". This plant is an angiosperm that belongs to the Asteraceae family characterized as a semi-herbaceous shrub. It originated from Central America and can be found mainly in tropical and subtropical regions of the Americas, Africa, and Asia (Ramírez-Riviera *et al.*, 2010; Silva *et al.*, 2021, GBIF..., 2023). It is found in Brazil's vacant lots, roadsides, and cultivated areas, demonstrating its good adaptation and rusticity (Silva *et al.*, 2021).

The literature addresses the morphophysiological responses of forages and their implications for plant management for other species (Zhang *et al.*, 2007; Tuffi-Santos *et al.*, 2015; Giltrap *et al.*, 2020). However, more information is needed on managing *T. diversifolia* during its growth. Information on the responses and ecophysiological adaptations along the growth of *T. diversifolia* is essential to infer stress conditions caused by the environment and to indicate growth and management conditions appropriate for the species. Studying the physiological aspects of *T. diversifolia* allows us to establish the capacity of solar energy capture, CO₂ fixation, and the synthesis of products necessary to support plant development and growth. Through these photosynthetic processes, atmospheric CO₂ is reduced to organic compounds essential to biomass production. The regrowth age modifies gas exchange, as there is

an increase in the leaf area index and higher interception of sunlight. The regrowth age is still a relevant factor in managing forage plants, as it influences the morphogenic indices, growth indices, and total biomass produced, determining the forage quality, depending on the leaf/stem ratio (Monção *et al.*, 2020). Despite the importance of ecophysiological studies on the management of forage plants, there are no studies in the literature on its physiology in different stages of regrowth and its relationship with the management of cutting *T. diversifolia*. Given the above, this study aimed to evaluate the gas exchange pattern and photosynthetic capacity of *T. diversifolia*, as well as the production of the dry mass of leaf and senescent material at different regrowth ages.

MATERIAL AND METHODS

The experiment was conducted in the region where the climate type is characterized as Savannah Tropical, according to the Köppen classification (Alvares *et al.*, 2013). The soil was classified as a Clay Silt Eutrophic Cambisol (Sistema..., 2013). Climate data during the days of evaluation is shown in Table 1. Planting of *T. diversifolia* was conducted using stem parts (cuttings) from plants approximately 2.0m high, horizontally distributed in planting furrows at a depth of 0.10m, with a spacing of 1.0 m between lines and 0.50m between plants. From planting until the beginning of the experimental period, two plant cuts were made to standardize clumps and increase the number of stems/plants.

Table 1. Temperature, relative humidity, and sunshine during the periods of evaluation

Item	Days after regrowth										Average
	21	28	35	42	49	56	63	70	77	84	
Tmin. (°C)	15.4	17.3	17.8	13.5	15.2	18.7	10.8	12.4	15.4	14.9	15.1
Tmax. (°C)	30.7	33.6	29.2	30.1	32.8	32.4	27.7	27.6	27.3	31.9	30.3
R.H. (%)	63.3	60.1	72.7	68.5	54.7	64.1	62.9	64.5	65.3	52.5	62.9

Tmin. – Minimum temperature; Tmax. – Maximum temperature; R.H. – relative humidity.

At the beginning of the experimental period, soil samples were collected in the 0 to 0.20m depth layer to determine soil fertility. The analysis indicated the following physicochemical characteristics: pH in water, 7.67; organic matter,

36.60g kg⁻¹; P, 1.88mg dm⁻³; K, 137.67mg dm⁻³; Ca, 7.07cmolc dm⁻³; Mg, 1.60cmolc dm⁻³; Al, 0.0cmolc dm⁻³; H + Al, 1.05cmolc dm⁻³; CTC, 10.06cmolc dm⁻³; base saturation, 89.67%; sand, 19.47%; silt, 38.80; clay, 41.73%.

Fertilization was used in this study according to the recommendations for sunflower cultivation. The experimental area was kept free of weeds and irrigated by the conventional sprinkler system to keep the soil in the field capacity.

The statistical design was completely randomized, with four replications and ten regrowth ages of *T. diversifolia*. The treatments consisted of evaluations performed at seven-day intervals, 21, 28, 35, 42, 49, 56, 63, 70, 77, and 84 days after the uniformity cut.

In all periods, physiological evaluations were performed using an Infrared Gas analyzer (IRGA), model LCA 4 (ADC - Analytical Development Company. Ltd, Hoddesdon, U.K.), in an open field with free air circulation. The evaluations were made in four plants chosen at random in the experimental plot. For assessment in each plant, three readings of the device were taken on three leaves of the upper third of each plant. The leaves chosen were fully expanded and apparently without symptoms of the disease. The values of each variable per plot were obtained by the average of the four selected plants for *T. diversifolia*. Assessments with the IRGA allowed obtaining the variables photosynthetic rate (A , $\text{CO}_2\text{m}^{-2}\text{ s}^{-1}$), stomatal water vapor conductance (g_s , $\text{mol m}^{-2}\text{ s}^{-1}$), transpiration rate (E , $\text{mol m}^{-2}\text{ s}^{-1}$), CO_2 concentration in the substomatal chamber (C_i , $\mu\text{mol mol}^{-1}$). From these data, we calculated the water use efficiency (WUE, $\mu\text{mol CO}_2\text{mol}^{-1}\text{ H}_2\text{O}$) by the ratio between the photosynthetic rate and the amount of water transpired.

During IRGA measurements, radiation, CO_2 concentration, and humidity were set at $1.200\mu\text{mol m}^{-2}\text{ s}^{-1}$, 340ppm, and 18mb, respectively, to minimize environmental variations. For more similar environmental conditions and correct data reading, the evaluations were performed between 9 and 11h. The last evaluation period, 84 days after the

standardization cut, refers to the phase when at least 50% of the plants emitted flowers.

The yield of leaves and senescent material was measured by successive collections and simultaneous physiological analyses. Based on the average height observed at each regrowth age, measured by measuring 20 plants from the experimental area, four plants were selected and cut at 0.40m from the ground, separated into parts (leaves, stem, and senescent material), and weighed. Drying was performed in a forced circulation oven at 60°C until constant weight. The values obtained were extrapolated to kg ha^{-1} of dry matter.

The data obtained were subjected to the Shapiro-Wilke normality test and homogeneity of variance using the O'Neill and Mathews test, meeting the assumptions for analysis of variance. Data were subjected to analysis of variance by the F test at 5% probability. When significant, regression analysis (5% probability) was performed according to regrowth age using the R Studio (R Core Team, 2023) statistical programs. Regression models were chosen based on the significance of the regression and determination coefficients.

RESULTS AND DISCUSSION

The regrowth ages of *T. diversifolia* influenced ($P \leq 0.05$) leaf gas exchange and plant growth. The photosynthetic rate, stomatal conductance, and water use efficiency of *T. diversifolia* presented a quadratic adjustment in the equation during the growth cycle, with the maximum observed value (inflection point) at 39, 51 and 47 days of regrowth, respectively (Figure 1). Immediately after the inflection points, the photosynthesis, stomatal conductance, and water use efficiency of *T. diversifolia* decreased markedly until the final evaluation period (Figure 1).

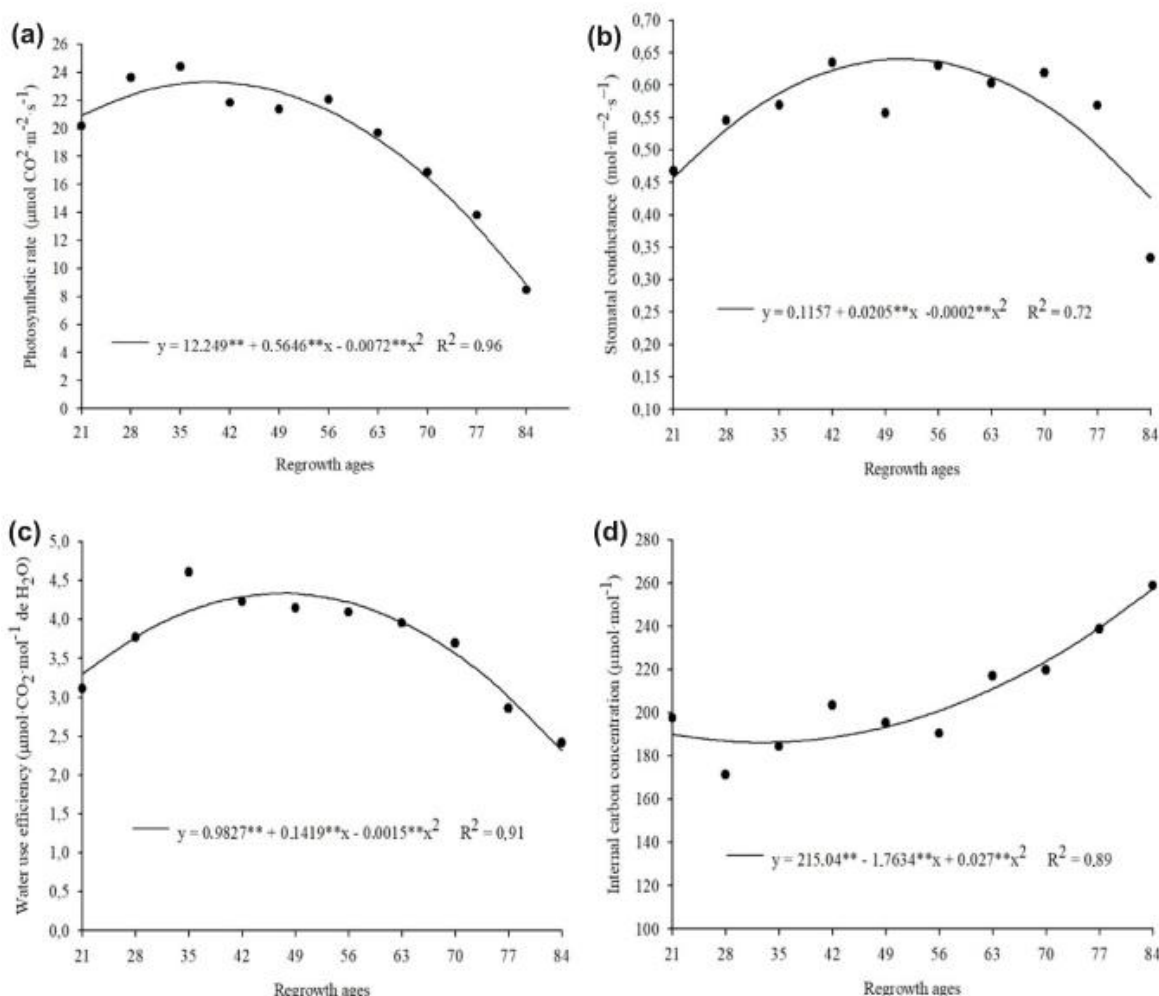


Figure 1. Photosynthetic rate (a), stomatal conductance (b), water use efficiency (c) and internal carbon concentration (d) of *Tithonia diversifolia* plants at different regrowth ages. ** Significant at 1% by T-test.

The photosynthetic capacity of the plant changes during its physiological development course. However, it is noted that during the early growth of *T. diversifolia*, the photosynthetic rate assumes relatively high values, even in the evaluation performed on days with high thermal amplitude (Table 1). In the current study, fully expanded leaves were evaluated. Fully differentiated young leaves increase their ability to produce photoassimilates continuously until reaching maturity (Taiz and Zeiger 2017) or shading by leaves of the upper extract. The

decline of photosynthetic rate is observed in old and senescent leaves, the result of chlorophyll degradation and degeneration of chloroplasts (Silva *et al.*, 2013). This effect can be seen in the decreases in photosynthetic rates in the final period of the crop cycle (Figure 1a).

Silva *et al.*, (2013) noticed higher photosynthetic rates in sunflower plants, a species of the same family as *T. diversifolia*, with a maximum value of $31.90 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 52 days after sowing. Likewise, Galon *et al.*, (2010) evaluated the

photosynthetic rate of sugarcane genotypes at 85 days after planting and noticed average values of $41.0 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$. Araújo *et al.*, (2010), studying irrigated Dwarf Elephant Grass, found photosynthetic rates between 15 and $22\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$, similar to the values observed in this study. It is noteworthy that all these studies were performed in the summer period, characterized by high temperature and radiation. In contrast, the present study was conducted in the winter period. The photosynthetic rates of plants are influenced by the environment (Costa *et al.*, 2020; Ferreira *et al.*, 2024), the species (Tuffi-Santos *et al.*, 2015), or even the genotype (Santos *et al.*, 2019). In the present study, the photosynthetic rate of *T. diversifolia* is also influenced by the age of the shoots (Figure 1).

Silva *et al.*, (2013) suggest that declines in photosynthetic rates are related to the partial closure of stomata, reflected by the decrease in stomatal conductance. Stomatal closure and the consequent reduction of CO_2 flow towards the carboxylation site negatively interfere with CO_2 assimilation by the photosynthetic apparatus. Thus, plant stomatal control is also directly related to the photosynthetic rate and the increase in biomass produced, but it is finely regulated due to water expenditure through transpiration. Some factors intrinsic to the plant affect stomatal control during individual growth and development, such as hormonal changes (Taiz and Zaiger, 2017) and morphological changes arising from the plant's growth phases (Ferreira *et al.*, 2022).

The water use efficiency adjusted to *T. diversifolia* regrowth periods (Figure 1c) showed a maximum value of $4.3\mu\text{mol CO}_2\text{mol}^{-1}\text{H}_2\text{O}$ at 47 days of regrowth. The most efficient use of water is related to a lower stomatal opening time, as this gap provides CO_2 absorption for photosynthesis and transpiration, which involves water loss (Ferreira *et al.*, 2022). Water use efficiency refers to the amount of carbon the plant fixes for each unit of water lost (Jaimez *et al.*, 2005). This information is crucial for managing *T. diversifolia*, especially in semi-arid regions or high-water usage systems. *T.*

diversifolia takes almost twice as much water to produce the same amount of biomass at 84 days of regrowth compared to its maximum efficiency point observed at 47 days after regrowth.

The CO_2 concentration in the substomatal chamber (C_i) changed significantly during growth (Figure 1d). When the photosynthetic rate is lower, the CO_2 level in the substomatal chamber tends to be higher (Concenço *et al.*, 2008). The probable degradation of the photosynthetic apparatus was responsible for the punctual accumulation of internal carbon in the leaf mesophyll (Figure 3). The behavior of stomatal conductance can also explain this; a decrease was observed from the 51st day of growth. Thus, considering only the conductance, a reduction of C_i in the final periods would be expected, which was not observed due to the decrease in photosynthesis.

The transpiration rate (E) measured in the ten regrowth ages of forage species showed a linear decrease with advancing age regrowth (Fig. 2). The opening and closing regulation of the stomata influence transpiration; the smaller the stomata's opening, the lower the transpiration rate due to the higher stomatal resistance. However, when observing the g_s (Figure 1b) and E (Figure 2) of *T. diversifolia* in the early regrowth ages (21 to 28 days), low g_s were observed. Besides, there was high transpiration due to the reduced canopy at this stage (Figure 3), which allows the action of winds and the removal of the boundary layer of air circulation on the leaf surface.

From the intermediate phase until the beginning of *T. diversifolia* flowering, the reduction of transpiration can be explained by the increase in stomatal resistance from the 51st day after regrowth, a period of low photosynthetic activity (Figure 1a) and that the plant seeks to save and improve water use efficiency. Microclimatic conditions imposed by the vegetation cover, such as lower wind speed and the deficit of vapor pressure on the leaf surface, may have contributed to a reduction in transpiration with the advance of regrowth ages.

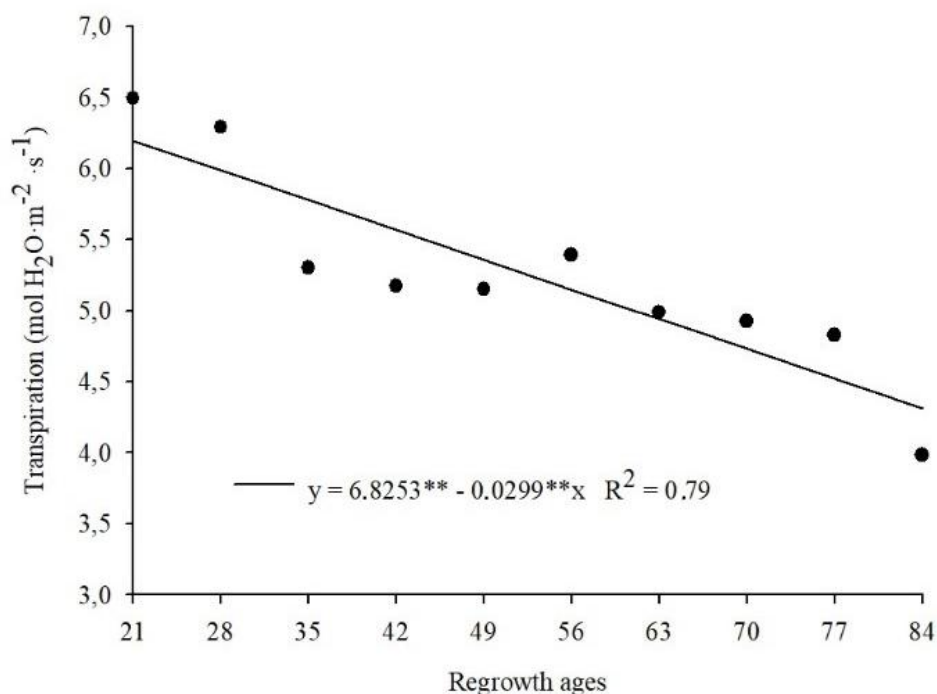


Figure 2. Transpiration of *Tithonia diversifolia* plants at different regrowth ages. ** Significant at 1% by T-test.

The *T. diversifolia* plants at different regrowth ages showed differences ($P \leq 0.05$) in the production of dry leaf mass, stem, and senescent material (Figure 3). The effect of regrowth age on leaf yield was adjusted to the quadratic model, and the maximum yield was recorded at 70.79 days ($5056.5 \text{ kg ha}^{-1}$). Plant senescence started at 35 days of regrowth (Figure 3), with a significant increase until the end of development. The intensification of the senescence process, especially of the basal leaves, can be attributed to self-shading. However, stem dry mass production increased linearly, with an increase of $289.43 \text{ kg ha}^{-1}$ for each regrowth day. Given these results, despite observing an increase in total forage production, added leaves, and stems, such information shows the compromise of the nutritional value of the plant due to the higher proportion of stem.

Considering the active leaf biomass, due to the difference between the dry mass of leaves and

the senescent material, the highest values were observed between 49 and 63 days of regrowth. So, although stem production has yet to reach high levels, this is the most suitable period for harvesting to utilize the nutritional quality of this forage. Despite the high proportion of stem in the material between 49 and 63 days after regrowth, given the plant's characteristics (Mauricio *et al.*, 2017), it was observed that they had a tender aspect at these times (field observation during cutting). Therefore, it is indicated for animal feed due to its high biomass production and nutritional value (Londoño *et al.*, 2019; Reis *et al.*, 2016). Harvesting of *T. diversifolia* before 49 days of regrowth would imply material of high morphological quality but low biomass yield (Figure 3). On the other hand, cutting the forage after 63 days of regrowth would lead to a progressive increase in stem and senescent material in the biomass (Figure 3) and low photosynthetic efficiency and water use (Figures 1a and 1c) by the plants.

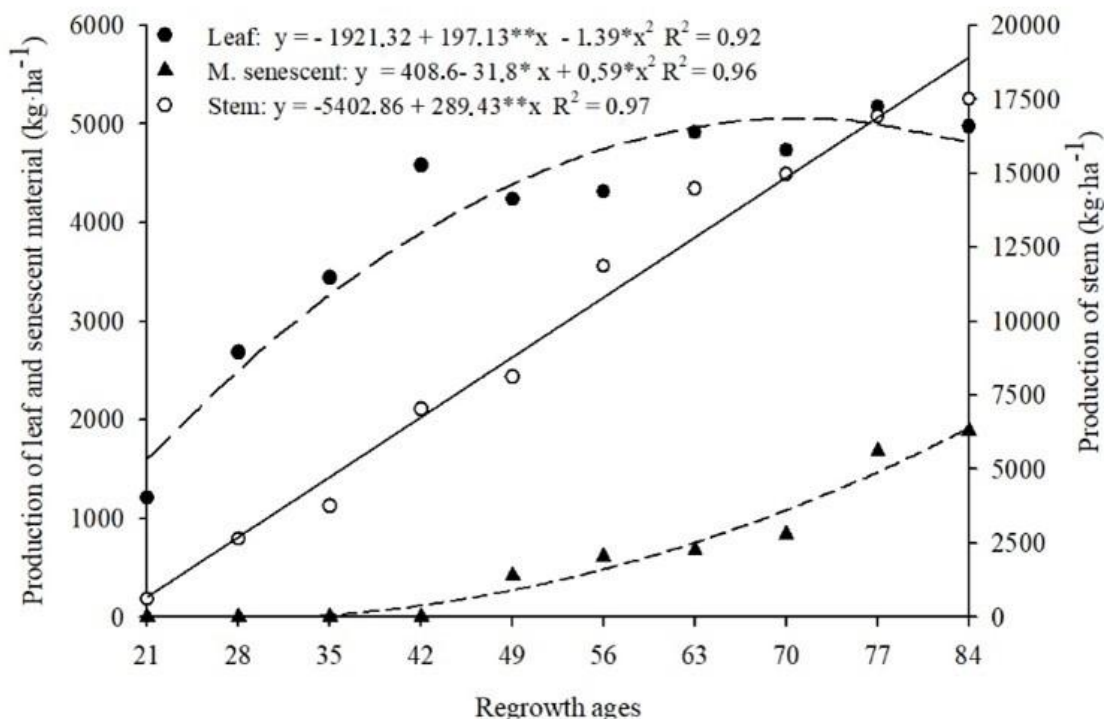


Figure 3. Dry mass production of leaves, stem, and senescent material of *Tithonia diversifolia* plants at different regrowth ages. ** Significant at 1% by T-test.

Such variations in leaf biomass and leaf senescence accumulation (Figure 3) allow the monitoring of the dynamics of effective photosynthetic production, resulting from the balance between photosynthesis and transpiration, which confirms the behavior of the physiological processes observed for *T. diversifolia*, mainly the limitation on carbon uptake by leaf tissues at the beginning of growth (Figure 1d). Considering the dynamic aspect of the environmental conditions and the growth and development of *T. diversifolia* at different regrowth ages, they suggest that such variations observed in gas exchange may also be associated simultaneously with the difference in climatic and ecophysiological factors. The ecophysiological evaluations related to the growth of *T. diversifolia* throughout its regrowth period allowed for a better understanding of the plant's behavior, which is fundamental for managing the species as forage.

CONCLUSIONS

A period of 15 days, between 49 and 63 days of regrowth, is recommended for using *T. diversifolia* as forage for cutting. This period is associated with good leaf availability, higher carbon fixation, greater water use efficiency, and little senescent material (values below 5% of the total dry mass of the plant) from *T. diversifolia* plants. Increasing age regrowth provides significant changes in gas exchange and photosynthetic capacity of the *T. diversifolia* with increments in dry matter production and increased leaf senescence. These changes imply a considerable variation in the water efficiency used by plants to be considered for the management of the species.

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