https://doi.org/10.22319/rmcp.v12i3.5720

Article

Phases of development and propagation of outstanding ecotypes of *Tithonia diversifolia* (Hemsl.) A. Gray

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Abstract:

The efficient propagation of plant species with high food potential plays a fundamental role in their adoption and use by producers. It is important, therefore, to develop economical and rapid methods for the successful establishment of more productive systems. In order to know some reproductive aspects of different outstanding genotypes of *T. diversifolia* and thus favor its propagation and improve its use in animal feeding, its germination potential, seed production and duration of the reproductive cycle were evaluated. Forty-two (42) plots were used in a randomized complete block design with seven genotypes of *T. diversifolia* and two levels (without and with fertilization). Genotypes had differences in all agronomic and seed production variables except for achene drying time and rudimentary seed percentage ($P<0.01$). In relation to the production of true seed, fertilization had a significant effect ($P<0.05$) and, in general,

increased the time of the phases evaluated. The highest seed production occurred in genotypes 7 and 5 and the percentage of germination had significant differences between genotypes and between pre-germinative treatments (*P*= 0.0001) with higher percentages in genotypes 3 and 6. It is concluded that despite the low viability of the true seed of *T. diversifolia*, there are genotypes with a greater potential for seed production and germination percentage, parameters that can be improved by means of pre-germinative treatments and the use of fertilizers.

Key words: Reproductive phase, Germination, Plant propagation, True seed, Seed viability.

Received: 26/06/2020

Accepted:28/10/2020

Introduction

The inclusion of high-protein forage plants with low fiber content in the diet of bovines results in an increase in the quality of forage biomass, which, in turn, results in an increase in milk and meat production[1,2]. For this reason, an adequate and efficient propagation should be an objective of study to improve their use.

Tithonia diversifolia (Hemsl.) A. Gray is a shrub of the Asteraceae family that, due to its ability to adapt to multiple environmental, edaphic and handling conditions, capacity for regrowth and rapid growth, and high value and nutritional contribution, has demonstrated its potential for animal feeding[3,4,5]. *T. diversifolia* can reproduce by both gamic seed and asexual seed, giving it great capacity for reproduction and colonization of new habitats[6,7]. This species flowers and produces seeds throughout the year, especially in October and November, although due to environmental conditions it can be of annual flowering[8,9]. However, some studies have reported germination percentages of less than 30 % under natural conditions[7,10].

Although field observations indicate that *T. diversifolia* has a great capacity to grow clonally[6], it is now known that material from true seed can favor the development of more extensive root systems, more vigorous plants, greater persistence of crops and faster recovery after cutting or grazing[11]. Nevertheless, it is still difficult to reach seminal material of good quality, and additionally, within this species there are genotypes with different germination capacities[11,12]. When studying the development of microsporogenesis, chromosomal abnormalities are recognized in 32 % of cells in metaphase I and anaphase I, identifying lagging chromosomes. On the other hand, the

reasons for the sterility of pollen in *Tithonia* could converge on the irregularities observed in its meiotic division[13].

In order to know some reproductive aspects of outstanding genotypes of *T. diversifolia* in Colombia and thus favor its propagation and better use towards animal feeding, the potential for germination, production of viable seeds and duration of the growth phases were evaluated.

Material and methods

Genotypes evaluated

Seven outstanding genotypes of *T. diversifolia*, for animal feeding, previously identified in a genetic diversity analysis[14] and collected at different sites in Colombia, were evaluated.

Location

The measurements were carried out in experimental plots located in the municipality of San Luis de Cubarral (Meta, Colombia), at 3°47'21.43"N, 73°49'15.93"W and at a height above sea level of 530 m. The site has an average annual rainfall of 4,100 mm, an average temperature of 24.8 ºC and is located in the tropical rainforest life zone (bh-T)[15].

Measurement of environmental conditions

During the entire sampling period, the environmental variables of precipitation (mm), temperature (ºC), relative humidity (%), solar radiation (W/m^2), dew point (ºC), wind speed (m/s) and THSW index (thermal sensation due to wind, relative humidity, irradiance [instantaneous solar radiation] and temperature [ºC]) were monitored by means of a Vantage Pro 2^{TM} (Davis ®) weather station.

Soil analysis

The chemical and physical variables of pH, E.C. (dS/m), field capacity (%), permanent wilting point (%), bulk density (g/cc), organic carbon (%), organic matter (%), texture, exchangeable potassium (mg/kg), exchangeable calcium (mg/kg), exchangeable magnesium (mg/kg), exchangeable sodium (mg/kg), exchangeable acidity (mg/kg), iron (mg/kg), manganese (mg/kg), copper (mg/kg), zinc (mg/kg), boron (mg/kg), phosphorus (mg/kg), sulfur (mg/kg) and the C.E.C. (meq/100 g), were determined in the soil where the experimental plots were located.

Growth and reproduction variables

The variables of duration of the vegetative phase (days), duration of the reproductive phase (days), duration of drying of achenes (days), flowering phase (days), flower heads per plant (#), seeds per flower head (#), seeds per plant (#), full seeds (%), empty seeds (%) and rudimentary seeds (%) were measured in the seven genotypes evaluated. The duration of the vegetative phase was determined from the moment of the uniformization cut in which the plants were pruned to 15 cm in height until the beginning of flowering of more than 50 % of the individuals that made up the experimental plots; the duration of the reproductive phase was from the moment of the appearance of flower buds until the fall of petals in 50 % of the plants; the duration of drying of achenes was from the fall of petals in the plants until achieving a dark brown color of flower heads, and the flowering phase was the sum of the vegetative, reproductive and achene drying phases. The variables of flower heads per plant (#), seeds per flower head (#), seeds per plant (#), full seeds (%), empty seeds (%) and rudimentary seeds (%) were measured manually and in five plants chosen at random from each of the experimental plots from the calculation of the sample size, assuming a maximum accepted estimation error and confidence level of 10 %. The crop evaluated was 13 months old and the measurements were made in the rainy season during the second period of 2019.

Germination of the true seed

Two pre-germinative treatments and one treatment without prior process (Treat 1) were evaluated. The pre-germinative treatments were: water at 80 ºC for 10 min (Treat 2)[16,17,18], and 50 % sulfuric acid (H_2SO_4) for 5 min (Treat 3)[19]. The seed was stored for four months after being collected with the aim of decreasing the physiological dormancy of the seed[17,20], and germination was evaluated in pots for 25 d using 50 seeds per site.

Experimental design and information analysis

The seven genotypes of *T. diversifolia* were established in experimental plots in a randomized complete block design with two factors (genotype and fertilization level). Each material had three repetitions which were made up of 36 plants each (0.8 m x 0.8 m) and two levels of fertilization (zero fertilization and fertilization according to the extraction of nutrients at 40 days of growth). According to evaluations, 40-day-old *T. diversifolia* plants extract 8.26 g of nitrogen, 4.3 g of potassium and 1.07 g of phosphorus[21]. This amount of nutrients was applied by fertilizing with urea (46 % of N), diammonium phosphate (DAP) ($(NH_4)2HPO_4$) (46 % of P_2O_5, 18 % of N) and potassium chloride (KCl, K_2O from 60 to 63 % and Cl from 45 to 47 %) at a rate of 16.22, 2.15 and 4.89 g/plant of urea, DAP and KCl, respectively. Germination evaluations were also analyzed under the same experimental design, assigning pots according to the plots arranged in the field. The mathematical model of the experimental design was:

$$yklj = \mu + \alpha k + \gamma l + \xi kl + \xi kl + \beta j + \varrho klj$$

Where:
yklj = Observation in the experimental unit of the variable to be evaluated;
μ = it is the mean of the general effect;
αk = effect of the factor k (collected materials 1, 2, 3...7);
γl = effect of factor l (fertilization level 1, 2...);
βj = effect of block j;
ξkl = interaction of the two factors;
ϱklj = random value, experimental error of the experimental unit lkj.

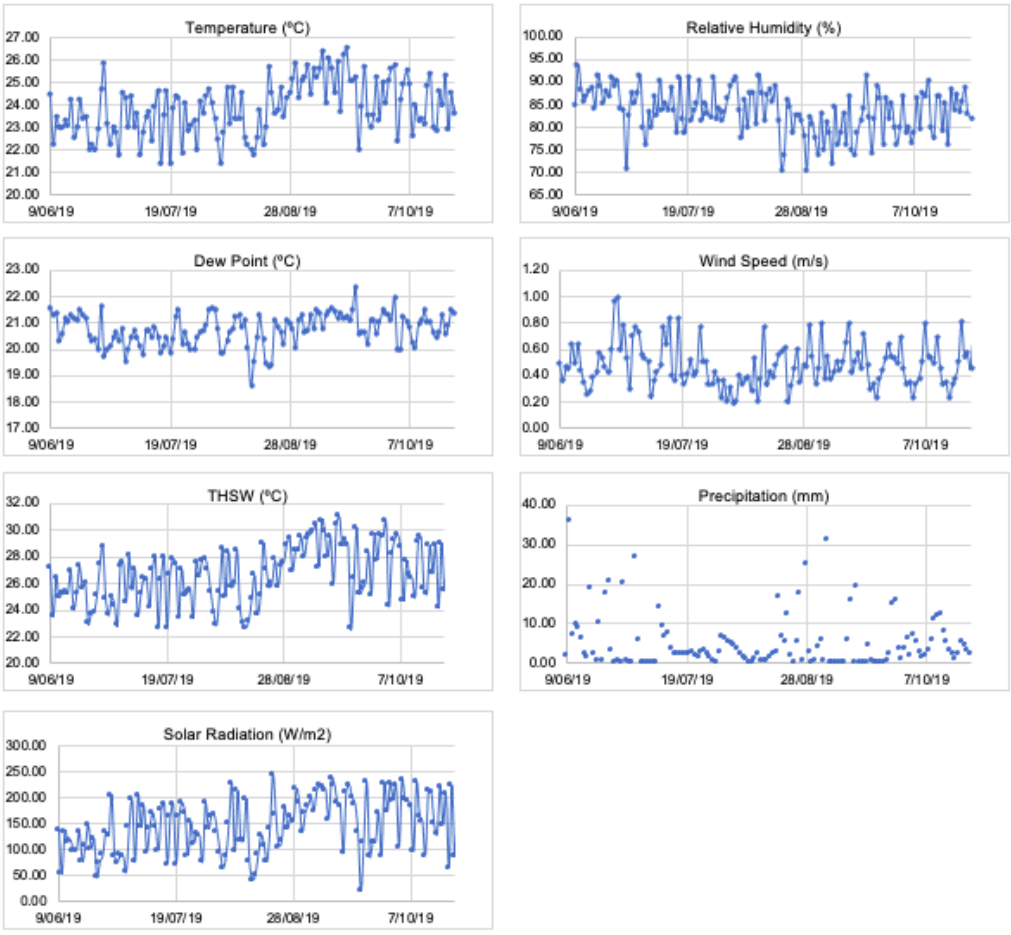
All analyses were performed in the RStudio tool using the library *"agricolae"*[22]. For the analyses, normality, homogeneity of variance and additivity were evaluated, in addition, when the difference between the means was identified, the Tukey contrast test was used, with a significance level of 0.05. Finally, in the variables of reproductive phase and full seeds, when the data sets did not meet the conditions for a parametric analysis, the Kruskal-Wallis and Mann-Whitney test was used for comparisons.

Results

Environmental conditions

Figure 1 shows the environmental conditions observed during the evaluation period. According to the records obtained at the experimentation site, the average temperature was 23.9 ± 1.2 ºC, the relative humidity was 83.4 ± 5 %, the average dew point was 20.8 ± 0.6 ºC, the wind speed was 0.7 ± 0.2 m/sec, the average THSW index was 26.7 ± 2.1 ºC, solar radiation 147 ± 53.3 W/m^2 and the accumulated precipitation was 556.2 mm.

Figure 1: Environmental conditions observed during the experimental period



The main chemical and physical characteristics of the soils where the experimental plots were located are shown in Table 1. The soils were acidic and presented low fertility due to limited supply of nutrients, characteristics usually found under tropical conditions.

Table 1: Chemical and physical characteristics of soil

Item	Block 1	Block 2	Block 3
pH	4.71	4.73	4.67
EC (dS/m)	0.07	0.06	0.06
Field capacity, %	18.5	15.8	13.8
Permanent wilting point, %	9.25	7.90	6.91
Bulk density, g/cc	1.51	1.46	1.44
Organic carbon, %	1.02	0.88	0.62
Organic matter, %	1.76	1.52	1.07
Sand, %	68	60	50
Silt, %	12	16	18
Clay, %	20	24	32
Texture	loam - clay - sandy	loam - clay - sandy	loam - clay - sandy
Exchangeable potassium, mg/kg	23.4	23.4	15.6
Exchangeable calcium, mg/kg	274	130	132
Exchangeable magnesium, mg/kg	24.0	16.8	18.1
Exchangeable sodium, mg/kg	23.2	18.4	16.1
Exchangeable acidity, mg/kg	218	219	172
Iron, mg/kg	305	358	459
Manganese, mg/kg	9.6	6.0	3.3
Copper, mg/kg	1.1	0.75	0.53
Zinc, mg/kg	0.7	0.4	0.4
Boron, mg/kg	0.11	0.17	0.13
Phosphorus, mg/kg	7.6	3.7	5.5
Sulphur, mg/kg	7.8	9.6	3.6
CEC, meq/100 g	3.64	3.35	2.82

pH= potential of hydrogen; EC= electrical conductivity; mg= milligrams; kg= kilograms; CEC= cation exchange capacity.

Development phases

The genotypes evaluated had significant differences in all the variables of development ($P<0.05$), except for the drying time of achenes and the percentage of rudimentary seeds ($P>0.05$) (Table 2). Genotype 1 was the material that had the longest time (140.1 d) in the sum of the growth and development stages (flowering phase) and genotype 4 was the one that presented the shortest times (127.2 d) with significant differences ($P<0.001$). In relation to the production of true seed, fertilization had an effect ($P<0.001$) by generating 2.14 times more seeds, and in general increased the time of the phases evaluated by approximately 5 d. Genotypes 5 and 7 were the materials with the highest production of

flower heads per plant, the highest percentage of full seeds and presented the highest number of seeds per plant, this probably associated with their higher number of branches. Similarly, the high percentage of empty seeds in all genotypes, as well as rudimentary seeds, is highlighted, which determine that more than 30 % of seeds do not have physical viability.

Germination

Table 3 presents the percentage of germination of the true seed of the genotypes evaluated with the three pre-germinative treatments used. According to the results obtained, there were significant differences between genotypes and treatments (P= 0.0001). On average, the germination percentages were 46.87, 53.53 and 25.97 for the control treatment, use of water at 80 ºC and 50 % sulfuric acid, respectively, and this process began three days after sowing.

Seed germination without treatment was significantly lower in genotype 1 compared to the other 6 genotypes (P<0.0001). In addition, this same material had the lowest values when the treatment with water and the treatment with sulfuric acid were used, although it did not have significant differences with genotypes 2 and 7. On average, fertilization increased the germination of the different genotypes by 9.2 % and the treatment that achieved greater germination was the use of water at 80 ºC and the treatment with less germination was the one that used sulfuric acid. In general, genotypes 3 and 6 were the ones with the highest percentage of germination, but only had significant differences compared to genotype 1.

Discussion

Knowing the phenology of shrub species and their potential for propagation allow not only to achieve greater use, but also a more efficient and economical use. The results of the times of each of the phases evaluated coincide with what was reported by Saavedra[23]. In general, the times of growth and development were modified by fertilization, probably due to the decrease of some stress factors. An adequate use of fertilization favors seed production and generates better development[24]. Saavedra[23], when evaluating three origins of *T. diversifolia*, found a very similar duration of the development process among the materials, with a duration of vegetative development between 88 and 137 d, the flowering process between 18 and 22 days, and a fruit formation between 13 and 18 d. As for the full, empty and rudimentary seeds, this same author found values between 65

and 75 %; 15.75 to 26 % and 9.31 to 12 %, respectively, similar to those of the present study.

Seed production for *T. diversifolia* has also been determined by different authors. In a study in Africa, it was found that this species can produce between 35 and 212 capitula per plant, between 32 and 62 seeds per capitulum, between 1,120 to 13,144 seeds per plant and that 1,000 seeds weigh between 6.42 and 7.5 g. These results show great variability in this species, and the results agree with those found in this evaluation, except for the number of seeds per capitulum, since in this study a higher number was identified[7].

Seed production was related to the number of branches, which favors a greater production of flower buds. The handling at the time of cutting, such as the height of pruning, favors a greater number of branches and biomass production[25,26]. *T. diversifolia* can reproduce by both gamic seed and asexual seed, which gives it great capacity for reproduction and colonization of new habitats[7,27]. This species flowers and produces seeds throughout the year, especially in October and November, although due to environmental conditions it can be annual[8,9]. Mature plants produce 80,000 to 160,000 seeds per square meter annually, of which 70 % fully develop, but germination percentages below 30 % under natural conditions have been reported[7,10], as occurred in genotypes 1 and 5 of the present study.

The results found in this study indicate that the germination of *T. diversifolia* during the first months of postharvest is acceptable, although variable between genotypes. Some authors highlight that this variability may be due to physiological dormancy[20] and irregularities observed in its meiotic division[10,13,28]. There are differences between ecotypes in their germination capacity. The results found coincide with a study of 29 materials collected in Cuba, where significant differences were found in the percentages of germination, which ranged between 5 and 67 %[29,30]. But the results are lower than reported in other studies where percentages greater than 70 % were reached in some materials[17,20]. Also, studies conducted in China demonstrate the variability of the gamic reproduction of this species in five regions of Yunnan Province[30]. In that study, the highest germination ranges (29.5 to 55.5 %) were determined with temperatures between 20 and 30 ºC. The results obtained coincide with those of the present study in that the greatest germination occurs between the first five to ten days for both conditions studied[30].

It has been identified that approximately 65 % of pollen grains lack sperm nuclei, indicating fertility close to 30 %[10]. Similarly, when studying the development of microsporogenesis, chromosomal abnormalities were recognized in 32 % of cells in metaphase I and anaphase I, identifying lagging chromosomes, and in metaphase II,

47 % of the cells presented asynchrony of a set of chromosomes and lagging chromosomes. It has been observed that in some species of dicotyledons the two nuclei resulting from the first meiotic division enter asynchronously in the second[31], which could result in male sterility, an anomaly that has also been related to spindle orientation, lagging chromosomes and anaphase bridges that affect the conformation of tetrads[32].

The presence of lagging chromosomes in anaphase I may be due to the lack of tension that the kinetochore sensory enzymes exert on the spindle forces, thus preventing the dragging of the chromosomes towards the poles, or to the fact that at least one of the chromosomes is misaligned, generating negative signals that the cell identifies[13].

It is also important to mention that there are divergent criteria on the viability and dormancy of *T. diversifolia* seed. It has been reported that seed storage and collection timing play an important role in the viability of the true seed of this species. Several authors reported that a storage for more than four months and a seed collection when the achenes are brown can increase the percentage of germination up to 90 %[25,33]. Other authors also report that pre-germinative processes, such as sulfuric acid and water at high temperatures for a few minutes (80 to 100 ºC), can increase germination[16,19] as those used in this work.

Improving the reproduction of this species via true seed would increase its potential as a forage shrub in livestock production systems. Field observations indicate that *T. diversifolia* has a great capacity to grow clonally[6], but at present it is known that material from true seed can favor the development of more extensive root systems, more vigorous plants, greater persistence of crops over time and faster recovery after cutting or grazing, although it is still difficult to reach seminal material of good quality[11].

In different studies, *T. diversifolia* has been considered as a forage shrub of high nutritional quality due mainly to its high contents of minerals (Ca and P), PC (>20 %), nonstructural carbohydrates and percentage of degradation (>70 %), and its low contents of NDF (<45 %) and ADF (<40 %)[3,4]. The PC values found in this species are as high or even higher than those observed in some tropical legumes such as *Stylosanthes guianensis* (20 %)[34], *Arachis pintoi* (19.7 %)[35] and *Gliricidia sepium* (18.23 %)[36], and are much higher than those observed in most tropical grasses, such as *Urochloa brizantha* (9.3 %)[3] and *Cynodon plectostachyus* (9.23 %)[37]. In addition, the NDF and ADF are lower than the common values observed for tropical forages[38], an aspect that probably does not limit voluntary consumption, the degradability of nutrients and their potential use by animals[1,3,4].

On the other hand, the consumption of *T. diversifolia* has been associated with increases in animal productivity and load capacity in the systems. In Colombia, the effect of this shrub under grazing conditions on the production and quality of bovine milk was evaluated and it was found that its consumption had significant effects on milk production, observing 9.70 and 15.4 kg milk/ha/day, respectively. In addition, the

production of protein, fat and total solids was also higher when the animals consumed *T. diversifolia* ($P<0.05$)[39]. Finally, the productive increase that has been obtained in systems with *T. diversifolia* favors lower emission intensities by reducing CH_4 emissions by enteric fermentation. The results did not find differences in daily emissions between conventional diets and diets with 25 % inclusion of *T. diversifolia* ($P= 0.351$), however, they identified differences in emissions per kg of weight gained in fattening animals ($P= 0.002$), going from 22.3 kg of CO_2-eq/kg of weight gained in the diet with brachiarias to 14.9 kg of CO_2-eq/kg of weight when the animals had access to *T. diversifolia*[40].

Conclusions and implications

T. diversifolia has genotypes with significantly different growth and development phases between them, modifying their reproductive moment and true seed production. According to the results found, there are genotypes with a higher production of viable true seed per plant, such as genotypes 5 and 7 evaluated in this study, which can be profiled as those most suitable to improve the propagation of this species. Although this species has a low germination (<50 %), there are pre-germinative processes with the ability to increase the percentage of germination such as the use of water at 80 ºC by 15 %. Also, the use of fertilizer increases not only the production of viable seed but also its germination, which is why it can be a viable alternative to treat seed-producing plants (20 % more germination). Finally, this study confirms that the species *T. diversifolia* has a high percentage of nonviable seeds, an aspect that invites to develop work aimed at understanding the factors responsible for this phenomenon in order to improve and facilitate its use, especially that of the outstanding genotypes identified in this work, since this species offers a high potential in animal feeding, product of its wide adaptation to different edaphoclimatic conditions, offer of high values of PC (>20 %), low content of ADF and NDF (<20 and 40 %, respectively), presence of different secondary compounds that modify the fermentative efficiency at the rumen level, and a high degradation that can be above 70 %.

Acknowledgements

To the *Sustainable Colombian Livestock* project, funded by the Government of the United Kingdom and the Global Environment Facility, for providing the necessary resources for the evaluations and collection of materials. Also, to the El Porvenir property for allowing evaluations to be carried out in its facilities, as well as to Minicienciasin its call for National Doctorates 727 of 2015.

Literature cited:

1. Barahona R, Sánchez S. Limitaciones físicas y químicas de la digestibilidad de pastos tropicales y estrategias para aumentarla. Cienc Tecnol Agropec 2005;6(1):69-82. https://dialnet.unirioja.es/servlet/articulo?codigo=5624715.

2. Schultze-Kraft R, Rao IM, Peters M, Clements RJ, Bai C, Liu G. Tropical forage legumes for environmental benefits: An overview. Trop Grassl 2018;6(1):1–14. http://www.tropicalgrasslands.info/index.php/tgft/article/view/394/241.

3. Ribeiro RS, Terry SA, Sacramento JP, Silveira S, Bento CB, Silva EF, *et al*. *Tithonia diversifolia* as a supplementary feed for dairy cows. PloS One 11 2016;e0165751. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5132235/

4. Mauricio RM, Calsavara LHF, Ribeiro RS, Pereira LGR, Freitas DS, Paciullo DS, *et al*. Feeding ruminants using *Tithonia diversifolia* as forage. JDVAR 2017;5(4):00146. http://medcraveonline.com/JDVAR/JDVAR-05-00146.php.

5. Rivera JE, Chará J, Gómez-Leyva JF, Ruíz TE, Barahona R. Variabilidad fenotípica y composición fitoquímica de *Tithonia diversifolia* A. Gray para la producción animal sostenible. Livest Res Rural Dev 2018;30, Article #200. http://www.lrrd.org/lrrd30/12/rive30200.html .

6. Ruiz TE, Febles G, Díaz H, Achang G. Efecto de la sección y el método de plantación del tallo en el establecimiento de *Tithonia diversifolia*. Rev Cubana Cienc Agr 2009;43(1):91-96. https://www.redalyc.org/pdf/1930/193015398016.pdf .

7. Obukohwo E, Umar B. Seed production, germination, emergence and growth of *Tithonia diversifolia* (HEMSL) A. Gray as influenced by different sowing depths and soil types. Am Eurasian J Agric Environ Sci 2014;14(5):440-444. https://www.idosi.org/aejaes/jaes14(5)14/9.pdf .

8. Pérez A, Montejo I, Iglesias J, López O, Martín GJ, García DE, Milián I, Hernández A. *Tithonia diversifolia* (Hemsl.) A. Gray. Pastos y Forrajes 2009;32(1):1–15. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-03942009000100001.

9. Chagas-Paula DA, Oliveira RB, Rocha BA, Da Costa FB. Ethnobotany, chemistry, and biological activities of the genus *Tithonia* (Asteraceae). Chem Biodivers 2012;9(2):210-235.

10. Alcorcés N, Lárez A, Mayz J. Adiciones al conocimiento citogenético de *Tithonia diversifolia* (Hemsl.) A Gray (Asteraceae). Acta Bot Venez 2007;30(2):267–275. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0084-59062007000200001.

11. Romero O, Galindo A, Murgueitio E, Calle Z. Primeras experiencias en la propagación de botón de oro (*Tithonia diversifolia*, Hemsl. Gray) a partir de semillas para la siembra de sistemas silvopastoriles intensivos en Colombia. Subtrop Agroecosystems 2014;17,524–528. https://pdfs.semanticscholar.org/3dff/1f62f0898f9df00e320078b4b8ba80864462.pdf.

12. Ruiz TE, Febles GJ, Alonso J, Crespo G, Valenciaga N. Agronomy of *Tithonia diversifolia* in Latin America and the Caribbean región. In: Savon LL, Gutierrez O, Febles F, editors. Mulberry, moringa and tithonia in animal feed, and other uses. Results in Latin America and the Caribbean. ICA / EDICA. La Habana, Cuba; 2017:171-20.

13. Taylor S, Scott M, Holland A. The spindle checkpoint: a quality control mechanism which ensures accurate chromosome segregation. Chromosome Res 2004;12:599-616. https://link.springer.com/article/10.1023/B:CHRO.0000036610.78380.51.

14. Rivera JE, Gómez-Leyva JF, Castaño K, Morales JG, Chará J, Barahona R. Diversidad molecular, química y morfológica en materiales de *Tithonia diversifolia* (hemsl.) Gray para la alimentación animal en Colombia y México. En: Chará J, *et al* editores. Congreso Internacional Sobre Sistemas Silvopastoriles. Manizales, Colombia. 2017:249–255.

15. Holdridge LR. Ecología basada en zonas de vida. Leslie Holdridge. IICA. San José, Costa Rica. 1986.

16. Akinola JO, Larbi A, Farinu GO, Odunsi AA. Seed treatment methods and duration effects on germination of wild sunflower. Exp Agric 2000;36:63–69.

17. Agboola DA, Idowu WF, Kadiri M. Seed germination and seedling growth of the Mexican sunflower *Tithonia diversifolia* (Compositae) in Nigeria, Africa. Rev Bio Trop 2005;54(2):395-402. https://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0034-77442006000200017

18. Nasreen S, Khan MA, Uddin S. Response of sunflower to various pre-germination techniques for breaking seed dormancy. Pak J Bot 2015;47(2):413-416.

19. Muoghalu JI, Chuba DK. Seed germination and reproductive strategies of *Tithonia diversifolia* (Hemsl.) A. Gray and *Tithonia rotundifolia* (P.M.) Blake. Appl Ecol Environ Res 2005;3(1):39-46. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.541.352&rep=rep1&type=pdf

20. Santos-Gally R, Muñoz M, Franco G. Efecto de la latencia sobre la germinación de *Tithonia diversifolia* (Asteraceae). En: Rivera J, *et al*, editores. Congreso internacional sobre sistemas silvopastoriles: por una producción sostenible. Libro de Actas. Editorial CIPAV, Cali. 2019:417–425.

21. Botero J. Potencial de extracción y utilización de nutrientes de *Tithonia diversifolia* y su relación con el rendimiento y calidad nutricional. [tesis doctorado]. Palmira, Colombia. Universidad Nacional de Colombia; 2017. http://bdigital.unal.edu.co/56938/2/2021-Julian_Mauricio_Botero.pdf.

22. RStudio Team. RStudio Version 1.1.447: Integrated Development for R. RStudio, Inc., Boston, MA. 2019. https://rstudio.com/.

23. Saavedra S. Fenología y fisiología de semillas de botón de oro *Tithonia diversifolia* (Hemsl.) Gray. [Tesis maestría]. Medellín, Colombia. Universidad Nacional de Colombia 2016 http://www.bdigital.unal.edu.co/55663/1/1128408177.2016.pdf

24. Márquez F, Sánchez J, Urbano D, Dávila C. Evaluación de la frecuencia de corte y tipos de fertilización sobre tres genotipos de pasto elefante (*Pennisetum purpureum*). Zootec Trop 2007;25(4):253-259. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0798-72692007000400003.

25. Partey SY. Effect of pruning frequency and pruning heighton the biomass production of *Tithonia diversifolia* (Hemsl)A. Gray. Agrofor Syst 2011;83:181–187.

26. Rodríguez I, Padilla C, Ojeda M. Características de la germinación de la semilla gámica de *Tithonia diversifolia* (Hemsl.) Gray y su comportamiento en condiciones de vivero. Livest Res Rural Dev 2019;31, Article #69. http://www.lrrd.org/lrrd31/5/idalma31069.html.

27. Ruíz TE, Febles G, Torres V, González J, Achan G, Sarduy L, Díaz H. Evaluación de materiales recolectados de *Tithonia diversifolia* (Hemsl.) Gray en la zona centro-occidental de Cuba. Rev Cubana Cienc Agríc 2010;44:291–296. https://biblat.unam.mx/hevila/Revistacubanadecienciaagricola/2010/vol44/no3/13.pdf

28. Rodríguez I, Padilla C, Torres V. Evaluación de tres métodos de poda de *Tithonia diversifolia* (Hemsl.) Gray bajo condiciones de pastoreo. Livest Res Rural Dev 2020; 32, Article #73. from http://www.lrrd.org/lrrd32/5/idal32073.html.

29. Ruiz TE, Febles G, Achan G, Díaz H, González J. Capacidad germinativa de semilla gámica de materiales colectados de *Tithonia diversifolia* (Hemsl.) Gray en la zona centro-occidental de Cuba. Livest Res Rural Dev 2018;30, Article #81. http://www.lrrd.org/lrrd30/5/ruiz30081.html.

30. Wang SH, Sun WB, Cheng X, Yang YM. Reproductive characteristics of *Tithonia diversifolia* and its geographical spread in Yunnan Province of South-West China. Acta Entomol Sin 2008;28(3). https://www.researchgate.net/publication/288293086.

31. González F, Rudall P, Furness C. Microsporogenesis and systematics of Aristolochiaceae. Bot J Linn Soc 2001;137:221-242. https://academic.oup.com/botlinnean/article/137/3/221/2557156 .

32. Mendes-Bonato AB, Junqueira R, Pagliarini MS, Valle CB Oliveira MI. Unusual cytological patterns of microsporogenesis in *Brachiaria decumbens* abnormalities in spindle and defective cytokinesis causing precocious cellularization. Cell Biol Int 2002; 26 (7):641-646. https://www.sciencedirect.com/science/article/abs/pii/S1065699502909297

33. Padilla C, Rodríguez I, Ruiz TE, Herrera M. Determinación del mejor momento de cosecha de semilla gámica, *Tithonia diversifolia* (Hemsl.) Gray. Livest Res Rural Dev 2018;30, Article #71. http://www.lrrd.org/lrrd30/4/idal30071.html.

34. La OO, González H, Vásquez M C, Estrada A. Composición química, digestibilidad ruminal in situ y fraccionamiento proteico de *Stylosanthes guianensis*. Ciencia en la frontera: revista de ciencia y tecnología de la UACJ 2018;15(2):29–35. http://erevistas.uacj.mx/ojs/index.php/cienciafrontera/article/view/2890.

35. Khan MT, Khan NA, Bezabih M, Qureshi M S, Rahman, A. The nutritional value of peanut hay (*Arachis hypogaea* L.) as an alternate forage source for sheep. Trop Anim Health Prod 2013;45:849–853.

36. Silva SF, Carneiro MS, Edvan RL, Pereira ES, Neto L, Pinto AP Camilo D. Agronomic characteristics and chemical composition of *Gliricidia sepium* grown under different residual heights in different seasons. Cienc Investing Agrar 2017;44(1):35-42.

37. Montoya E, Barahona R, Chará J. The nutritional balance of early lactation dairy cows grazing in intensive silvopastoral systems. Ciênc Anim Bras 2017;18:1-12. https://www.scielo.br/scielo.php?script=sci_arttext&pid=S1809-68912017000100218.

38. Souza LF, Mauricio RM, Gonçalves L C, Saliba EOS, Moreira GR. Produtividade e valor nutritivo da *Brachiaria brizantha* cv. Marandu em um sistema silvipastoril. Arq Bras Med Vet Zootec 2007;59(4):1029-1037. https://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-09352007000400032.

39. Rivera JE, Cuartas CA, Naranjo JF, Tafur O, Hurtado EA, Arenas FA, Chará J, Murgueitio E. Efecto de la oferta y el consumo de *Tithonia diversifolia* en un sistema silvopastoril intensivo (SSPi), en la calidad y productividad de leche bovina en el piedemonte Amazónico colombiano. Livest Res Rural Dev 2015;27, article #189. http://www.lrrd.org/lrrd27/10/rive27189.html.

40. Molina IC, Donneys G, Montoya S, Villegas G, Rivera JE, Chará J, Barahona R. Emisiones in vivo de metano en sistemas de producción con y sin inclusión de *Tithonia diversifolia*. Congreso Nacional de Sistemas Silvopastoriles y Congreso Internacional de Sistemas Agroforestales. Puerto Iguazú, Misiones, Argentina, 2015:678-682.

Table 2: Phases of agronomic development and true seed production of different genotypes of *Tithonia diversifolia*

Parameter	Treatments-Genotypes							Fert		Ge	Fert	SEM
	1	2	3	4	5	6	7	No	Yes	*P-value*		
Vegetative phase, days	86.8^{a}	84.2^{a}	80.6^{ab}	74.3^{c}	76.2^{bc}	80.7^{ab}	76.3^{bc}	77.1	82.4	<0.001	<0.001	0.92
Reproductive phase, days	31.8^{a}	29.2^{ab}	28.8^{ab}	31.5^{ab}	29.3^{ab}	27.5^{b}	31.3^{ab}	29.9	30	0.022	0.845	0.4
Drying of achenes, days	21.8	21.7	22.7	21.5	23.3	23.3	21.7	22.7	21.7	0.67	0.134	0.36
Flowering phase, days	140^{a}	135^{ab}	132^{bc}	127^{c}	129^{bc}	131^{bc}	129^{bc}	130	134	<0.001	0.001	0.91
Flower heads per plant, #	32.1^{b}	62.9^{a}	46.1^{ab}	53.6^{ab}	70.1^{a}	45.1^{ab}	71.9^{a}	36.3	72.7	0.002	<0.001	4.14
Seeds per flower head, #	142^{c}	155^{ab}	149^{bc}	152^{ab}	153^{ab}	156^{ab}	164^{c}	147	159	<0.001	<0.001	1.59
Seeds per plant, #	4,668^{c}	10,112^{ab}	6,958^{bc}	8,238^{abc}	10,908^{ab}	7,217^{bc}	11,946^{a}	5,460	11,697	<0.001	<0.001	713
Full seeds, %	63.5^{b}	69.5^{a}	62.3^{b}	71.3^{a}	69.3^{a}	67.0^{ab}	62.7^{b}	65.5	67.5	0.025	0.244	0.91
Empty seeds, %	23.1^{ab}	22.8^{ab}	25.0^{a}	20.0^{b}	20.8^{b}	22.8^{ab}	25.7^{a}	21.5	24.3	0.041	0.006	0.56
Rudimentary seeds, %	13.5	7.67	12.7	8.83	9.83	10.2	11.7	11.1	10.2	0.059	0.418	0.56

Fert= fertilization; Ge= genotypes, SEM= standard error of the mean.
^{abc} Different letters in the same row denote difference (P<0.05).

Table 3: Percentage of germination of true seed of different genotypes of *Tithonia diversifolia*

Tr	Genotypes (Ge)							Fert		Ge	Fert	SEM
	1	2	3	4	5	6	7	No	Yes	*p- value*		
1	32.6^{b}	45.4^{a}	54.1^{a}	45.8^{a}	47.7^{a}	53.9^{a}	48.6^{a}	42.6	51.1	0.001	0.002	1.52
2	39.5^{b}	51.6^{a}	58.2^{a}	54.1^{a}	52.9^{a}	62.2^{a}	56.9^{a}	46.9	59.7	0.001	0.001	1.89
3	17.5^{c}	22.3^{abc}	31.9^{a}	30.3^{ab}	19.7^{c}	31.8^{a}	26.9^{abc}	23.7	30.2	0.001	0.025	1.31

Tr=Treatment; SEM= standard error of the mean; Fert= fertilization; Tr 1= without prior treatment; Tr 2= water at 80 ºC for 10 min; Tr 3= immersion in 50 % sulfuric acid.
^{abc} Different letters in the same row denote difference (P<0.05).