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Physiological quality of *Tithonia diversifolia* (Hemsl.) A. Gray seeds as a function of harvest period and storage conditions

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Abstract

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Tithonia diversifolia is a shrub of the *Asteraceae* family native to Central America and found on all continents. It has multiple functions with emphasis on use in animal feed and as a medicinal plant. It is usually propagated vegetative, with little information on seed propagation. For this reason, the aim of this study was to define the ideal time for harvesting the seeds and to evaluate the quality of the seeds according to time and storage condition. Therefore, achenes were collected on different days after the anthesis (14, 16, 18, 20, 22, 24, 28, 32, 36 and 40 days) of inflorescence. Following harvesting, the following variables were evaluated: seed dry matter mass, germination percentage, germination speed index, germination speed and percentage of full achenes. Moreover, these variables were analyzed as a function of two storage conditions (refrigerated and non-refrigerated environment) and two storage times (6 and 12 months). The ideal harvest period comprised the period from 28 to 36 days after anthesis, in which seeds with germination percentages exceeding 75% and percentage of full achenes of 100% were obtained. The chilled environment provided the highest percentages of seed germination. Moreover, germination speed increased throughout the storage time. The seeds showed a high percentage of germination after 12 months of storage, both in refrigerated and non-refrigerated conditions.

Keywords: Asteraceae; medicinal plant; Mexican sunflower; multipurpose shrub; non-conventional forage; seed production

Introduction

Tithonia diversifolia (Hemsl.) A. Gray is a species of the *Asteraceae* family and native to Central America, being found on all continents (Winnifred & Morris, 2014). It is popularly known as daisy, buttercup, honey flower, or Mexican sunflower. It is a shrub that can reach 1 to 3 m in height and has multiple functions, especially for use in animal feed and as a medicinal plant. It is forage with a protein content of up to 28% and a digestibility of 65% (Peters et al., 2011) and is recommended as a food component for goats (Wambui, 2006) and dairy cattle (Ribeiro et al., 2016).

Moreover, it is used in traditional medicine in several countries to treat diabetes, malaria, snakebite, measles, gastric ulcers, menstrual pain and wounds (Ajao & Moteetee, 2017). Its extracts have an anti-trypanosomal effect (Sut et al., 2018) and its essential oil was active against *Staphylococcus aureus* (Orsomando et al., 2017). In guinea pigs it improved immune response and reduced cholesterol and triglycerides (Ejelonu et al., 2017). It also has potential for use in plant phytosanitary control (Pulido et al., 2017; Zhao et al., 2017).

Even though there is some information, lack of knowledge of simple techniques for sexual propagation and the poor supply of cuttings (asexual propagation) are the main

obstacles to use of this species (Medina et al., 2014). Therefore, the need for research evaluating factors that can affect seed quality and that are relevant to obtain high quality seeds becomes evident. In this context, the aim of this study was to evaluate the physiological quality of *T. diversifolia* seeds as a function of harvesting time, storage condition and time.

Material and Methods

Cultivation of parent plants

Cuttings of 20 cm in length and of herbaceous origin were collected from individuals occurring spontaneously in the municipality of Viçosa (20°45′34.29″ S and 42°52′28.97″ W, altitude of 633 meters), state of Minas Gerais, Brazil. From the cuttings, 12 plants were cultivated with spacing of 4 m x 4 m, in three rows containing 4 plants each. Planting was carried out at the *Universidade Federal de Viçosa* experiment area, in October 2015 at the beginning of the rainy season (Figure 1). For the planting, 30 cm × 30 cm × 30 cm ditches were used and 5 liters of chicken manure, 200 g of dolomitic limestone and 30 g of NPK (10-10-10) were used.

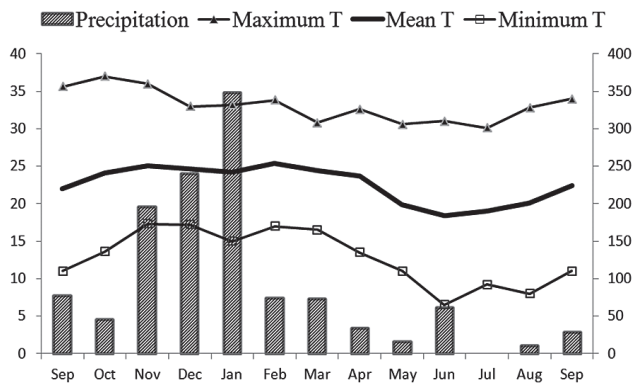

Fig. 1. Monthly weather chart from the weather station located in the Universidade Federal de Viçosa showing the variables precipitation (mm), maximum temperature (°C), average temperature (°C) and minimum temperature (°C). Period from September 2015 to September 2016.

Source of data: National Institute of Meteorology

Experiment 1: Maturing the seeds

In the flowering stage of *T. diversifolia*, from April to July 2016, inflorescences were marked at the anthesis phenological stage (Figure 2-A), using colored strands. The inflorescences were protected with organza sacks to protect against attack by birds (Figure 2-B). Inflorescences were collected at 14, 16, 18, 20, 22, 24, 28, 32, 36 and 40 days post anthesis (DAA). Inflorescences without flower shoots (SFR) an undefined number of days after anthesis, treatment denominated SFR, totaling 11 treatments were collected. SFR was proposed considering ease of visual identification of the inflorescence at this stage, which could facilitate harvesting if it possessed good-quality seeds.

Fig. 2. *A* – Inflorescence opening; *B* – Protective sacks; *C* – Thermoelectric stove drying with forced air circulation; *D* – Flower head after removal of seeds

After harvesting, the flower heads were dried in an air circulation oven at a temperature of 25°C for 120 hours (Figure 2-C), and the seeds were later removed by manually turning them upside-down without the need to clean through sieving.

The following evaluations were performed:

– Mean number of seeds per flower head: calculated by counting the number of seeds obtained in a sample consisting of 10 flower heads collected at random.

– Percentage of full achenes in the periods 14 DAA, 22 DAA, 32 DAA, 40 DAA and SFR. For each period, 8 x-ray images were taken; each image with 8 seeds was considered a replication. The Faxitron X-RAY Corporation, model MX 50/LX60 with tray height of 10 cm, exposure time of 18 seconds and power 26 KV was used.

– Number of inflorescences foraged by birds: this was determined in flower heads collected at 14 DAA, 16 DAA, 18 DAA, 20 DAA and 22 DAA. For each harvesting, 100 inflorescences were marked at the anthesis stage and were not protected with an organza sack, with this made it possible to quantify the percentage of inflorescences attacked.

– Dry matter mass per seed: determined for all harvest times following the methodology of Brazil (2009), with 4 replications per lot, each plot containing 100 seeds.

– Percentage, germination speed index and germination speed: the seeds from each harvest were treated with Derosol fungicide (6 drops of the product for 250 seeds). Then the seeds, in six replicates of 40, were distributed on two sheets of germitest paper moistened with an amount of water equivalent to 2.5 times the weight of the dry paper, in germination boxes. The boxes were covered and packed in a germination chamber (Mangelsdorf type) at 25°C. Germinated seeds were counted daily until germination stabilization occurred at 53 days. Seeds with radicle protrusion were considered germinated, and the percentage was calculated.

The germination speed index was calculated as suggested by Marguire (1962):

$$IVG = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \frac{G_n}{N_n}.$$

The germination speed was calculated according to Edmond & Drapala (1958):

$$VG = \frac{(N_1 G_1) + (N_2 G_2) + \ldots (N_n G_n)}{G_1 + G_2 + G_N}.$$

When: G is the number of seeds germinated in each daily count and N is the number of days after sowing until each count day

– Statistical analysis: the experiment on seed maturation had a completely randomized design with 11 treatments (14, 16, 18, 20, 22, 24, 28, 32, 36 and 40 DAA + SFR) and six replications, each plot with 40 seeds. After variance analysis, regression analysis was performed at 5% of probability.

Experiment 2: Storing the seeds:

For the 16 DAA, 24 DAA, 32 DAA and 40 DAA harvest periods, part of the seeds were packed in 240 mL PET bottles, occupying 50% of the volume of each bottle. These bottles were stored for 6 and 12 months in two conditions: refrigerated at 5°C and 45% UR and laboratory condition at 25°C and UR of 70%, which temperatures were kept constant through air conditioning.

At time 0 (soon after harvest) and 6 and 12 months after harvest, underwent the germination test as described above.

The seed storage experiment had a completely randomized design and triple factorial scheme (four harvest times – 16 DAA, 24 DAA, 32 DAA, 40 DAA), three storage times of 0, 6 and 12 months , two storage conditions (refrigerated and non-refrigerated environment) and six replicates, each plot having 40 seeds. Variance analysis was performed, followed by the Tukey test at 5% probability to compare the means of storage times and days after anthesis for the following variables: percentage of germination, germination speed index and germination speed. Regression analysis was also performed to evaluate the functional relationship between days after anthesis and the variables evaluated. All analyses were carried out using R software.

Results and Discussion

Seed maturation and harvesting

T. diversifolia flowered between April and July 2016, the months with the lowest rainfall and temperature indices in the municipality of Viçosa, MG (Figure 1). Each inflorescence of *T. diversifolia* produced on average 189.8 seeds, a number close to that found by Muoghalu & Chuba (2005) (179.7 seeds), but higher than that found by Etejere & Olayinka (2015) (32-62 seeds), Muoghalu (2008) (136 – 144 seeds) and Silva et al. (1999) (89.9 seeds).

The inflorescence is composed of flowers shoots and tubular flowers. The tubular flowers, important for the production of achenes, mature in the centripetal direction, that is, from the edge of the flower head towards the center (Figure 3). This fact is relevant and may result in variations in seed quality from flower heads collected at the same time.

According to the variance analysis, there was a significant difference at 5% probability between the harvest times for the seeds for the variables: percentage of full achenes, percentage of germination, germination speed index, and germination speed and seed dry matter mass. The regression

Fig. 3. Phenological conditions of the inflorescence of *T. diversifolia.* The tubular flowers (central part of the flower head) open from the edge toward the center, centripetally (*a* to *e*). At about 5 DAA (*c*) more than 50% of the flowers have already opened and at 7 DAA (*e*) all have already opened and the petals begin to wither. From 8 to 9 DAA (*f* – *g*) the petals are greatly reduced and at 10 DAA (*h*) are no longer present. From 10 DAA to 30 DAA (*l*), the tubular flowers become darker.
Source: Dr. Paulo Hilst.

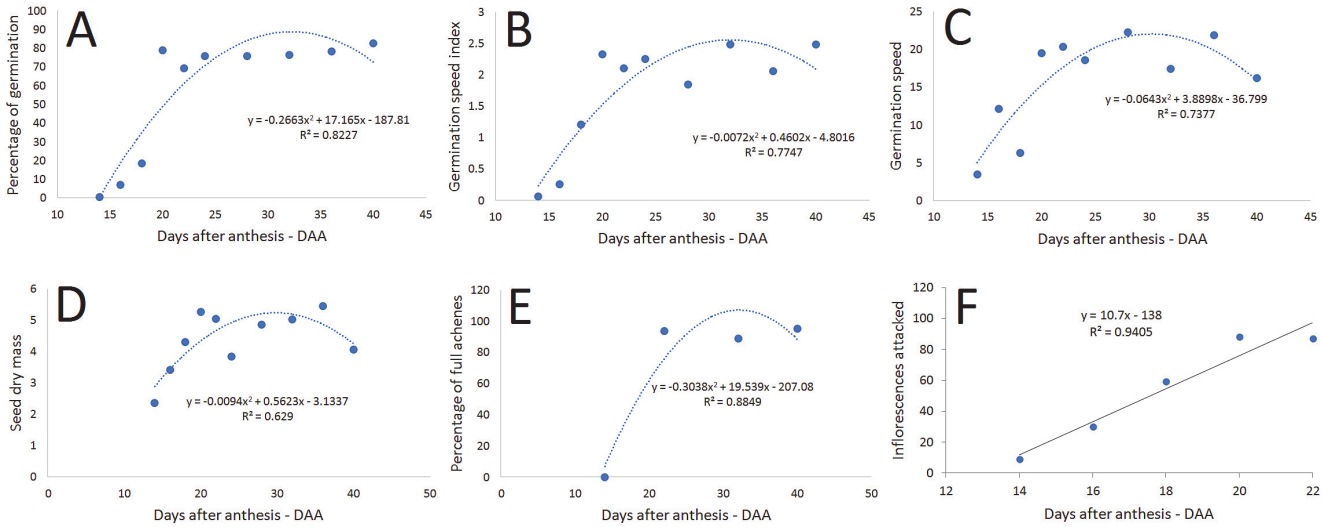

Fig. 4. Percentage of germination, germination speed index, germination speed (days), seed dry mass (mg/seed), percentage of full achenes and inflorescences attacked (%) according to harvest time (days after anthesis – DAA)

Table 1. Equations adjusted to estimate the ideal day for collection for the different variables evaluated in the maturation experiment, coefficient of determination (R^2), days after anthesis, which gives the highest values for the variables evaluated (DAA with maximum y) and maximum point of the equation (y maximum)

Variable	Adjusted equation	DAA with maximum *y*	R^2	Maximum *y*
Percentage of germination	$-0.2663x^2 + 17.165x - 187.81$	32.23	0.82	88.79
Germination speed index	$-0.0072x^2 + 0.4602x - 4.8016$	31.96	0.77	2.55
Germination speed	$-0.0643x^2 + 3.8898x - 36.799$	30.25	0.74	22.03
Seed dry mass	$-0.0094x^2 + 0.5623x - 3.1337$	29.91	0.63	5.28
Full seeds	$-0.3038x^2 + 19.539x - 207.08$	32.16	0.88	100

equations were adjusted to evaluate the functional relationship between the harvesting season and the response variables (Figure 4 and Table 1).

Foraging of unprotected inflorescences by birds of the *Psittacidae* family was observed. There was a linear increase from 14 to 22 DAA, and at 22 DAA about 90% of the inflorescences had been foraged (Figure 4-F), indicating that, as in sunflower cultivation, the presence of birds is an important factor for planning harvest, and the use of desiccants to reduce exposure time is recommended for this (Bolsón, 1981).

An increase was found in dry matter content during seed development (Figure 4-D). The highest values were observed at harvest times between 28 and 32 DAA, in which the highest value was at 30 DAA (Figure 4-D and Table 1). At 40 DAA there was a reduction in dry matter, which can be explained by the respiration process of the still moist seeds, consuming part of the accumulated reserves. This variable is important, considering that higher accumulated dry mass is an indication that the seeds have reached physiological maturity, when there is no more translocation of assimilates from the plant to the seeds (Marcos Filho, 2015). From that moment, the seeds of orthodox species begin the process of drying until reaching compatible water content for harvest. However, it is worth mentioning that the coefficient of determination of the regression was reduced (R2 = 0.63), and the periods of 24 and 40 DAA are discrepant. Something that attracts attention in the species and which explains this variation is the fact that the inflorescences and the achenes differ greatly in size and this will, consequently, result in variations in the mass of dry matter of the achenes.

With regards the percentage of full achenes (Figure 4-E), there is an increase in values throughout the maturation process, as also observed for the dry matter of the seeds, with maximum values between 28 and 32 DAA, and the highest value for 32 DAA (Figure 4-E and Table 1). These results are confirmed by the image analysis performed with the seeds of each harvest period (Figure 5). It can be seen that seeds

harvested at 14 DAA and the SFR had empty glumes. For the SFR harvest period, starting from 10 DAA (Figure 3), no good quality seeds were obtained, as there were low germination percentages (6.5%) and low values for seed dry matter mass (3.2 mg).

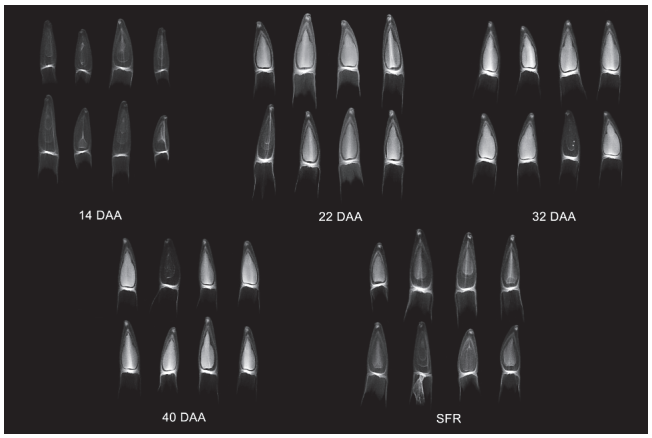

Fig. 5. X-ray image of achenes at different times of harvesting

There was an increase in germination percentage, germination speed index and germination speed throughout seed development (Figure 4-A, B and C). At 14 DAA, germination was practically nil, increasing in the next crops until reaching higher values between 28 and 36 DAA, with a slight drop at 40 DAA, which was also observed for seed dry matter. The highest value for germination percent occurred at 32 DAA with 88% (Table 1). In an experiment conducted in Colombia in which seeds without control of the harvesting season were harvested, germination percentages lower than 10% were obtained (Porras, 2016). Etejere & Olayinka (2015) obtained emergence percentages of 76.76% in seeds planted on the soil surface, however the work did not detail at what time the flower heads were harvested.

In general, germination percentage, germination speed index and percentage of full achenes were highest at harvesting between 28 and 36 DAA (Figure 4-A, B and F). It is worth mentioning that IVG expresses the mean number of seeds germinated per day and that the percentage of full achenes indicates satisfactory seed development, therefore, the higher the means of these variables, the better the seed quality. For sunflowers, full seed maturation was achieved between 30 and 40 days after flowering, which was established when 2/3 of the tubular flowers were in anthesis (Maeda et al., 1987). In Calendula, another species of the *Asteraceae* family, seed physiological maturity occurred between 28 and 32 DAA, and seeds harvested at 36 DAA showed greater vigor (Silveira et al., 2002).

The lowest means for germination speed were obtained with harvests at periods 14 DAA, 16 DAA and 18 DAA (Figure 4-C). This variable expresses the number of days required for germination and the lower the value the greater the seed vigor. However, in this work, germination speed results for these earlier harvest times are associated with the fact that germination stabilized within a few days after sowing (18, 20 and 15 days, respectively) and for this reason the values obtained were low. Thus, the germination process was fast, but the final percentage of germination was low.

Although high germination percentages were obtained without using techniques to overcome dormancy in this study, other researchers indicated humid heat and light as treatments for overcoming dormancy in the species (Akinola et al., 2000; Agboola et al., 2006). The use of moist heat is associated with overcoming physical dormancy which occurs in seeds with a tegument that hinders absorption of water, also known as hard seeds. In addition to the genetic factor, environmental conditions during maturation influence the proportion of these seeds impermeable to water (Rolston, 1978). It is likely that, for this reason, dormancy was found in the works cited but was not observed in this study. This makes sense considering that the ecological explanation for the existence of hard seeds includes the ability to recolonize burned areas and bear ingestion by animals and birds, and delayed germination and dormancy are common in species adapted to light (Rolston, 1978; Vazquez – Yanes & Segovia 1984), all characteristics related to daisies. It is worth noting that during the germination test in this study, those seeds that did not germinate were hard, and no seeds were found with fungi or softness, characteristics associated with dead seeds.

The achenes harvested at 14, 16, 18 DAA and SFR, all with low percentage of germination, were grayish in color (Figure 6) while achenes harvested from the 22 DAA onwards were brownish in color. It can be seen from the results in Figure 4 that seed quality was higher from 22 DAA onwards and that before this both germination and dry matter content were low. Therefore, seed coloration can be used as a morphological descriptor to aid selection of viable seeds; similar to what happens for calendulas, as when the seeds change in coloration from green to cream it is an indication of their good viability (Silveira et al., 2002).

According to the results obtained, to obtain viable seeds, it is recommended to harvest inflorescences without petals, with

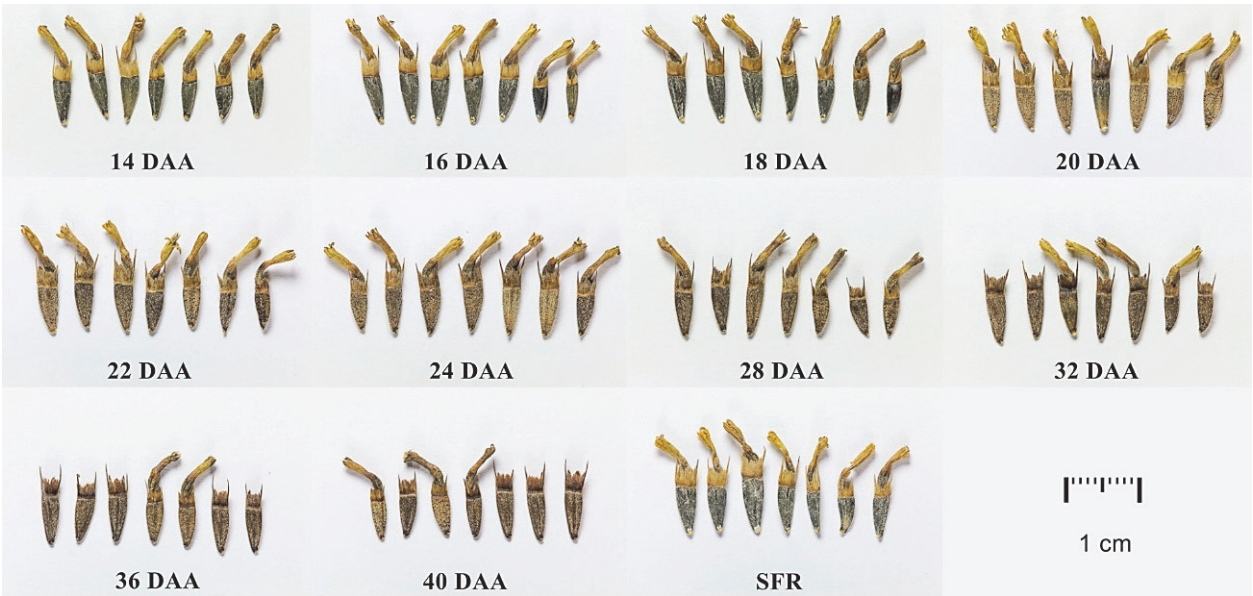

Fig. 6. *T. diversifolia* achenes collected at different stages of maturation
Source: Dr. Paulo Hilst.

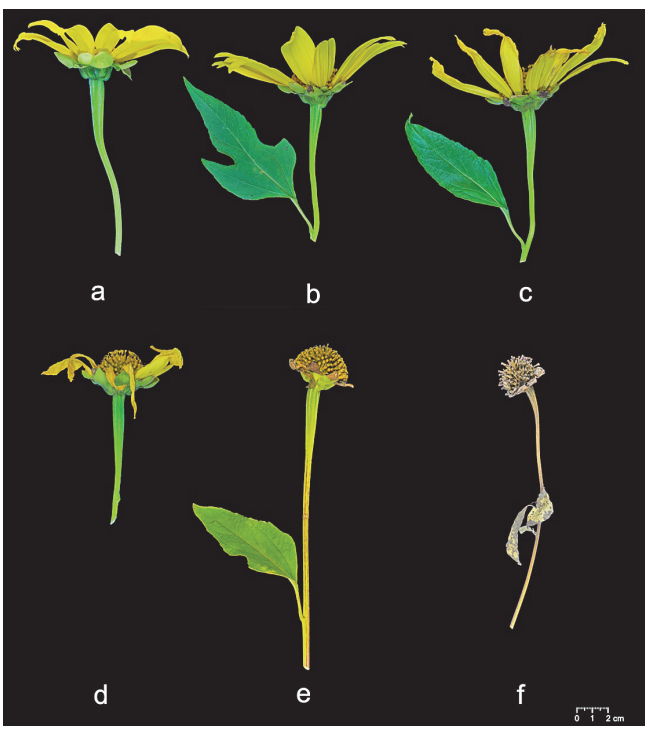

Fig. 7. Phenological stages of the inflorescence of *T. diversifolia*. Inflorescences with petals (*a* – *c*) from 1 DAA to 6 DAA.Reduction of petals at 8 DAA (*d*). From 22 DAA the inflorescence begins to dry (*e*) and at 30 DAA with completely dry inflorescence (*f*).
Source: Dr. Paulo Hilst

receptacle almost or totally brownish, brown bracts and dark tubular flowers (Figures 3 and 7), conditions similar to what has been recommended for seeds of other species of the same family. For *Smallanthussonchifolius* (Asteraceae), it is recommended to harvest achenes when the flower head is brown in color (Ibañez et al., 2017) and for sunflowers when the bracts turn yellow and brown and when much of the flower head begins to turn brownish too (Schneiter et al., 1981).

Considering the variables analyzed, it is found that to obtain higher quality daisy seeds, the harvest should occur between 28 and 36 DAA, particularly due to the percentage of germination, germination speed index, percentage of full achenes and seed dry matter mass. These variables are significant technological parameters that characterize the process of seed maturation (Marcos Filho, 2015) and help determine the ideal harvest point.

Quality of seeds according to harvest period, time and storage condition

According to the variance analysis (Table 2), for the variables germination percentage and germination speed, there was a significant difference at 5% probability between levels of the factors storage time, storage condition and harvesting period. For the germination speed index variable only, there was no significant difference for the storage condition factor.

The regression equations were adjusted to evaluate the functional relationship between harvest period, time and storage condition, and response variables (Figure 8).

Table 2. Summary of the variance analysis for the variables germination percentage (GERM), germination speed index (IVG) and germination speed (VG) as a function of harvesting periods, time and storage condition of *T. diversifolia*

Source of Variation	GL	Mean Squared		
		GERM	IVG	VG
Storage time	2	365.97*	22.59*	612.10*
Storage condition	1	563.08*	0.88ns	88.60*
Harvest period	3	44885.60*	141.58*	499.01*
Time x Condition	2	2.63ns	0.26ns	0.16ns
Time x Period	6	207.60*	6.19*	25.50*
Condition x Period	3	114.82ns	0.87ns	21.77*
Time x Condition x Period	6	119.99ns	1.46*	10.14ns
Residue	120	77.76	0.39	7.40
Total	143			

*ns significant and not significant, respectively, at 5% probability by the F test

For the three variables studied (GERM, IVG and VG), there was no significant interaction at 5% probability between the factors storage time and storage condition. However, there was a significant interaction between the factors of storage time and harvest period (Table 2). Only for the germination speed was there significant interaction at 5% probability between the factors storage condition and harvest period. For germination speed index, there was a significant interaction between the three factors (Table 2). As the interactions were significant, they were developed.

Table 3 shows that the highest percentages of germination occurred in harvests at 32 and 40 DAA and with 6 and 12 months of storage (Table 3 and Figure 8). On these harvest days, the best germination rates were obtained with 6 and 12 months of storage (Table 3), and Figure 8 clearly shows the tendency of this variable to reduce over time.

Comparing germination obtained at different harvesting periods (Table 3), it was observed that there was no sig-

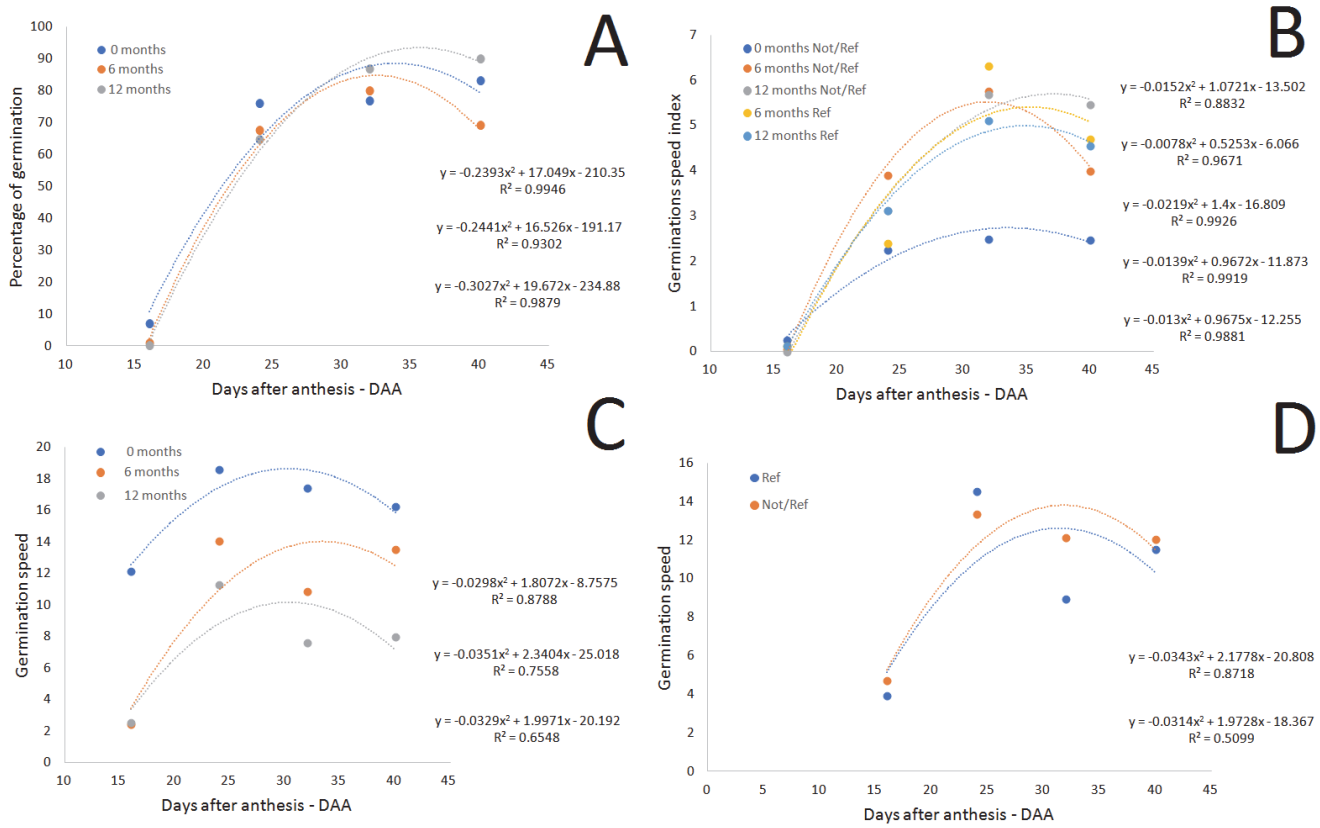

Fig. 8. *A*. Germination percentage according to harvest periods and storage time; *B*. Germination speed index according to harvest periods, storage time and condition; *C*. Germination speed according to harvest periods, storage time and *D*. Germination speed according to harvest periods and storage condition

Table 3. Means for germination percentage and germination speed at different harvest periods (DAA) and storage times (months)

Period (DAA)	Percentage of germination			Germination speed (days)		
	Storage times			Storage times		
	0 months	6 months	12 months	0 months	6 months	12 months
16	7.0 bA*	1.2 cA	1.2 cA	12.1 bA	2.4 cB	2.5 cB
24	75.8 aA	69.1 bA	73.5 bA	18.5 aA	14.0 aB	11.2 aB
32	76.6 aB	83.9 aAB	88.5 aA	17.4 aA	10.8 bB	7.6 bC
40	82.9 aAB	73.7 bB	86.6 aA	16.2 abA	13.5 abA	7.9 bB
CV%	5.01			27.63		

*Means followed by identical lower case letters in the columns or upper case in the lines do not differ according to the Tukey test at 5% probability.

nificant difference between seeds harvested at 24, 32 and 40 DAA at storage time 0. At six months, germination was higher for seeds harvested at 32 DAA, which was significantly higher than the germination obtained at other harvesting periods. At 12 months, greater germination was obtained for seeds harvested at 32 and 40 DAA.

In general, the lowest germination values were observed at 16 DAA, which can be attributed to seed immaturity, with low dry matter content (Figure 4). This figure shows that seeds at 32 DAA showed higher values for dry matter, with a slight reduction at 40 DAA.

The germination values obtained at 12 months storage for harvests from 24 DAA, 32 DAA and 40 DAA show that daisy seed quality was maintained over time. As observed in this work with daisy seeds, Maeda et al. (1987) found that sunflower seeds maintained high percentages of germination for up to 24 months of storage in uncontrolled laboratory conditions.

For all harvest periods, the germination speed reduced as a function of storage time (Table 3 and Figure 8), as already highlighted above. Considering the periods with the highest germination (24, 32 and 40 DAA), the germination speed index increased as a function of storage time (Table 4 and Figure 8). These data show that storage time may favor seed quality. It is noteworthy that certain variables related to seed quality can be favored during storage. Muoghalu & Chuba (2005) reported that the germination of *T. diversifolia* improved four months after harvesting and Agboola et al. (2006) indicated that the seeds appear to require some post-ripening period that favors maturation. Seeds of *Carthamus tinctorius* L. (Asteracea) and sunflower seeds overcame dormancy throughout storage (Maeda et al. 1987; Oba et al. 2017).

It is known that the period of a few months of storage, also called post-maturity is a usual procedure for overcoming dormancy, which can be explained: due to structural and chemical changes in the seeds, reduced demand for light, alteration in the balance between abscisic acid and gibberellin, increasing the temperature range favorable to germination, reduced need for nitrate, and increasing the speed of germination (Marcos Filho, 2015). In the study, the response of *T. diversifolia* to storage is likely associated with an increase in the speed and intensity of imbibition as a function of structural changes, even though there was not a great increase in the percentage of germination. Such evidence is valid considering that during the germination tests many hard seeds were observed for the species, as already mentioned.

Table 4. Mean of germination speed index at different harvest periods (DAA), storage conditions with (C/REF) and without (S/REF) refrigeration and storage times (0, 6 and 12 months) and germination speed means at different harvest periods (DAA) and storage conditions with (C/REF) and without (S/REF) refrigeration

Period	IVG						VG	
	S/REF			C/REF			C/REF	S/REF
	0	6	12	0	6	12		
16	0.2 bA	0.0 cA	0.0 cA	0.2 bA	0.1 dA	0.1 cA	3.9 cA	4.7 bA
24	2.2 aB	3.9 bA	3.1 bA	2.2 aB	2.4 cB	3.1 bA	14.5 aA	13.3 aA
32	2.4 aB	5.7 aA	5.6 aA	2.4 aC	6.3 aA	5.1 aB	8.9 bB	12.1 aA
40	2.4 aC	4.0 bB	5.4 aA	2.4 aB	4.7 bA	4.5 aA	11.5 bA	12.0 aA
CV%	55.9						27.6	

*Means followed by lower case letters in the columns and upper case in the lines do not differ according to the Tukey test 5% probability; S/REF = without refrigeration and C/REF = with refrigeration

The percentage of germination in the refrigerated condition was higher compared to the non-refrigerated condition (Table 2), with averages of 62.2% and 58.4%, respectively. A similar situation was observed in sunflowers, which maintained a high percentage of germination in environments with reduced temperature and relative humidity (Lima et al, 2014). The situation observed is coherent considering that lower temperature reduces the speed of chemical reactions decreasing the rate of deterioration of orthodox seeds (Marcos Filho, 2015).

For germination speed index, there was a significant interaction at 5% probability between storage time and condition (Table 2). After the development, differences between the two storage conditions were found only when the harvests were at 40 DAA and stored for 12 months.

In the non-refrigerated condition, the highest rates of germination were obtained in seeds harvested at 32 DAA and stored for 6 and 12 months and at those harvested at 40 DAA and stored for 12 months (Table 4). This situation is justified considering that the storage time can help overcome dormancy, as reported above.

For germination speed, only in the DAA 32 harvest was there a significant difference between the storage conditions, and the refrigerated condition favored germination speed and presented the best mean of the means for harvest days with a high percentage of germination (Table 4).

The results of the research showed potential for use in seed propagation for *T. diversifolia*. However, further work should be undertaken as little information is available on the subject.

Conclusions

T. diversifolia seeds with higher germination and dry matter mass were obtained when harvesting was between 28 and 36 DAA. Through the X-ray test, no empty seeds or glumes were detected at these harvesting periods.

The refrigerated environment provided the highest percentages of seed germination. Moreover, germination speed decreased and germination speed index increased over the storage time. The seeds showed a high percentage of germination after 12 months of storage, both in the refrigerated and non-refrigerated conditions.

T. diversifolia seeds harvested at 14, 16, 18 DAA and inflorescences without petals were grayish in color and had a low percentage of germination. From 22 DAA, the seeds were brownish in color. To obtain viable seeds, it is recommended to collect inflorescences without petals, with receptacle almost or totally brownish, brown bracts and dark tubular flowers.

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