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Effect of storage time and pre-germination treatments on nine native herbaceous species with the potential to restore degraded soils of La Primavera Forest, Jalisco

Efecto del tiempo de almacenamiento y tratamientos pre-germinativos en nueve especies herbáceas nativas con potencial para restaurar suelos degradados del Bosque La Primavera, Jalisco

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Abstract
Background: Percentage and speed of germination are crucial factors that can restrict processes involved in plant succession during ecological restoration. Pre-germination treatments and the effect of storage time on germination percentage and mean germination time were investigated in nine native herbaceous plants of the La Primavera Flora and Fauna Protection Area.
Question: Do the seeds of the studied species go dormant, and can storage time affect germination percentage and mean germination time?
Species studied: *Aeschynomene villosa* var. *longifolia* (Micheli) Rudd, *Crotalaria pumila* Ort., *Dalea leporina* (Ait.) Bullock, *D. foliolosa* (Ait.) Barneby, *Desmodium aparines* (Link) DC., *D. distortum* (Aubl.) JF Macbr., *D. tortuosum* (Sw.) DC., *Paspalum notatum* Flüggé, and *Zornia reticulata* I. E. Smith.
Place and dates of study: Area of Protection of Flora and Fauna La Primavera. From 2019 to 2020
Methods: Under greenhouse conditions, four treatments were applied prior to germination: scarification with a tweezer pliers, immersion in water at 40 °C for 24 and 48 h, and control.
Results: Seven species had highly viable (≥ 90 %) seeds after 12 months of storage. Five species with seed dormancy reached germination above 80 % after 24 months of storage and scarification with a tweezer pliers. Mean germination time for most species decreased with the scarification treatment.
Conclusion: Our findings provide basic information on the germination of native species that could help restore degraded sites in the La Primavera Flora and Fauna Protection Area.
Key words: Dormancy, germination, germination time, seeds, storage, viability.

Resumen
Antecedentes: La germinación de semillas y la velocidad de germinación podrían ser factores que restringen los procesos de sucesión de plantas en la restauración ecológica. Se investigó el efecto de tratamientos de germinación y tiempo de almacenamiento en el porcentaje de germinación y el tiempo medio de germinación de nueve herbáceas nativas del Área de Protección de Flora y Fauna La Primavera.
Pregunta: ¿Las semillas de las especies estudiadas tienen latencia y puede el tiempo de almacenamiento afectar el porcentaje de germinación y el tiempo medio de germinación?
Especies estudiadas: *Aeschynomene villosa* var. *Longifolia* (Micheli) Rudd, *Crotalaria pumila* Ort., *Dalea leporina* (Ait.) Bullock, *D. foliolosa* (Ait.) Barneby, *Desmodium aparines* (Link) DC., *D. distortum* (Aubl.) JF Macbr., *D. tortuosum* (Sw.) DC., *Paspalum notatum* Flüggé y *Zornia reticulata* I. E, Smith.
Lugar y fechas de estudio: Área de Protección de Flora y Fauna La Primavera. De 2019 a 2020
Métodos: En invernadero, con cuatro tratamientos: escarificación con pinzas alicata, inmersión en agua a 40 °C/24 horas y por 48 horas, así como el control.
Resultados: Siete especies tuvieron altos porcentajes de viabilidad (≥ 90 %) en semillas con 12 meses de almacenamiento. Cinco especies alcanzaron germinación superior al 80 % con 24 meses de almacenamiento y escarificadas con alicata. El tiempo medio de germinación para la mayoría de las especies disminuyó con el tratamiento de escarificación con alicata.
Conclusión: Nuestros hallazgos brindan información básica sobre la germinación de especies nativas que podrían ayudar a restaurar sitios degradados en el APFFLP.
Palabras clave: Almacenamiento, germinación, latencia, semillas, tiempo de germinación, viabilidad.

The Area of Protection of Flora and Fauna La Primavera (APFFLP) is the main forest area that provides ecosystem services to the city of Guadalajara (De la Mora-De la Mora & López-Miguel 2022). It is the main habitat for 961 species of vascular plants, of which 59 are orchids. There are also 29 species of mammals and 135 species of birds (SEMARNAT 2000). Unfortunately, the APFFLP is affected by forest fires, between 0 and 60 events per year in the last 15 years, with severe impacts on vegetation cover (Huerta-Martínez & Ibarra-Montoya 2014) resulting in areas with severe erosion (Tecuapetla-Gómez *et al.* 2021).

Plant establishment by seed is a restrictive process, especially in degraded soils (Romo-Campos *et al*. 2009, Kildisheva *et al*. 2020). Seed germination process are crucial factors that can restrict processes involved in plant succession during ecological restoration (Sánchez *et al*. 2019). Germination is affected by internal factors, such as physiological and morphological development of the embryo and its potential to germinate (viability), as well as by external factors such as water availability, temperature, and light (Baskin & Baskin 2004). Specifically, in the establishment of native species with the capacity to improve degraded soils, non-viable or dormant seeds that do not germinate can be an obstacle (Jiménez-Alfaro *et al*. 2016, Baskin & Baskin 2020). Few studies have contributed to understanding germination mechanisms of native herbaceous species that could facilitate ecological restoration processes (Kiss *et al*. 2018), especially in Mexico (Godinez-Álvarez & Flores-Martínez 1999).

One of the challenges of ecological restoration is the selection of native species that can colonize degraded soils and generate suitable environments to favor secondary succession (Meli *et al*. 2014). For example, higher and faster germination rates could benefit seeds by allowing them to establish prior to other species and thus evade competition (Mayo-Mendoza *et al*. 2018).

Breaking seed dormancy is essential for ecological restoration because it increases success in the establishment of plants that will initiate the processes of secondary succession (Baskin & Baskin 2020). However, many native species have dormancy mechanisms that have not been elucidated (León-Lobos *et al*. 2020). Therefore, it is necessary to identify pre-germination treatments to accelerate their establishment by direct seeding (Mayo-Mendoza *et al*. 2018).

Plant species of the families Fabaceae and Poaceae have great potential to be used in ecological restoration programs (Baskin & Baskin, 2004, Jayasuriya *et al*. 2013). Many of these plants are pioneers in the ecological succession process and facilitate the establishment of other plants (Mandoni *et al*. 2013). Species of Fabaceae are commonly used in restoration of disturbed habitats because they increase soil fertility through nitrogen fixation. However, seed dormancy has been detected in many species of these families, limiting their potential for use in restoration of disturbed habitats (Jayasuriya *et al*. 2013). Seeds of many Fabaceae species have physical dormancy because they have an impermeable hard coat that prevents the embryo from absorbing water. This type of dormancy can be broken by chemical or mechanical scarification, or by immersion in hot water, among other methods (Baskin & Baskin 2004). Seeds from several Poaceae have physiological dormancy (Baskin & Baskin 2004). However, seed dormancy is also controlled by the impermeability of lemma and palea (structures that cover the seeds) (Ellis *et al*. 1985).

Seed storage time also affects seed germination. Seed germination in some species diminishes with aging, while seeds from other species can remain viable over time, and in other species aging breaks seed dormancy (Flores & Jurado 2011). This knowledge is critical for effective seed storage after harvesting for restoration practitioners and native seed producers, as it is key to maintaining seed viability (De Vitis *et al.* 2020).

Mexico has a wealth of diverse native plants that are potentially important resources for restoring degraded soils (León-Lobos *et al*. 2012). The APFFLP has undergone severe loss of vegetation cover due to fires and overgrazing, among other causes (De la Mora-De la Mora & López-Miguel 2022). For this reason, identifying species with potential for ecological recovery is needed. This research aimed to assess the effect of storage time and germination treatments on nine herbaceous native (which are very abundant in degraded sites of the APFFLP) and was guided by the following questions: Do the seeds of the studied species have dormancy? Can storage time affect germination percentage and mean germination time?

Materials and methods

Study area. The study was conducted in the Ejido Emiliano Zapata, municipality of Zapopan, Jalisco, Mexico, coordinates 103° 35' 35.37" W and 20° 42' 00" N, in the Area of Protection of Flora and Fauna La Primavera (APFFLP) located in the Transverse Neovolcanic Belt of the state of Jalisco. Its altitude varies between 1,400-2,200 m, and approximately 50 % of the area has slopes steeper than 44 %. Regosol soils are found in 92 % of the protected area; these soils are shallow and poor in organic matter (less than 2 %) (SEMARNAT 2000), and there are acidic extrusive rocks (SEMARNAT 2000). The predominant climate is temperate sub-humid (C(w1)(w), with summer rains; average annual precipitation is 900 mm and the average annual temperature is 20.6 °C (García 1973). The vegetation types are oak-pine forest, oak, pine, gallery forest, and tropical deciduous forest (Rzedowski 1978).

Seed collection. In the APFFLP, during September and October 2019 and 2020, seeds were collected from mature fruits of at least 10 adult individuals by species of *Aeschynomene villosa* var. *longifolia* (Micheli) Rudd, *Crotalaria pumila* Ort, *Dalea leporina* (Ait.) Bullock, *D. foliolosa* (Ait.) Barneby, *Desmodium aparines* (Link) DC., *D. distortum* (Aubl.), *D. tortuosum* (Sw.) DC., *Paspalum notatum* Flüggé and *Zornia reticulata* I. E. Smith. Collected fruits were dried and cleaned; the seeds were manually extracted and preserved in brown paper bags. Samples were stored well ventilated at the Seed Laboratory of the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) at room temperature (25 °C) for two years (seeds collected during 2019) and for one year (seeds collected during 2020).

Seed viability test. Seed viability and vigor of 30 seeds of each species and quantified using tetrazolium (2,3,5-triphenyl tetrazolium chloride) following Maldonado *et al*. (2016). Seeds were completely immersed in water in sealed plastic bags for 24 h at 25 °C ± 1 °C under dark conditions in a germination chamber (SEEDBURO, MPG1000). The seed coat or tegument of hydrated seeds was manually removed with a tweezer pliers and the embryos were extracted. These were covered with a 0.1 % tetrazolium solution in Petri dishes and placed inside the chamber for 24 h. Then, the tetrazolium solution was removed, and the seeds were carefully washed with abundant tap water. Seed viability was evaluated with a stereomicroscope (VELAB, VE-S3) and classified according to embryo coloration (ISTA 2010). Seeds were considered viable when an intense reddish or pinkish color was observed in the embryo and non-viable when the embryo did not show any apparent coloration or only portions of it were colored.

Embryos were sorted by color: live with high vigor (totally stained intense red), live with low vigor (pale red coloration or discolored sections), and non-viable (colorless). Viability was expressed as the percentage of live embryos of the total number of evaluations for each species.

Imbibition curves. Under laboratory conditions, 30 seeds per species were individually weighed on an analytical balance (Ohaus Pioneer PA214), each seed was placed in a glass with 25 ml of distilled water at room temperature (24 °C). After 12 hours, the seeds were drained and dried with an absorbent paper towel and weighed again to determine their weight; this was repeated every 12 hours until completing 36 hours when constant weight was obtained. The amount of water imbibed (ml) by the seeds was determined by the difference between initial weight and final weight. The weight obtained in g was transformed into volume units (1 g of increased weight = 1 ml imbibed).

Germination tests. In April 2021, germination tests were performed in a greenhouse located at CUCBA (20° 44' 53.6"N - 103° 30' 52.2" W). Seeds were disinfected with 1 % sodium hypochlorite for 3 seconds and rinsed with distilled water. Three pre-germination treatments were established: scarification with a tweezer pliers (P), immersion in distilled water at 40 °C for 24 hours (IW24) and for 48 hours (IW48) (Water bath: Yamato, Bain Marie 100, USA, 5~90 °C), and the control. Two seed lots with different storage times were used: seeds collected in 2020 (1-year-old seeds) and seeds collected in 2019 (2-year-old seeds). We did not have seeds with zero months of storage (fresh seeds) in this experimental design, because we did not have access to the greenhouse during the seed collection season.

Seeds were sown individually in polystyrene containers 58.8 × 32.7 cm, with 60 round cavities 15 mm in diameter and 181 ml volume. The substrate used was "jal" (vitreous acid volcanic rock or pumicite) (SEMARNAT 2000), which was sifted with a 2 mm diameter sieve and sterilized in an oven (JISICO Co., Ltd. J-DECO) at 120 °C. For each treatment, 100 seeds divided into five experimental units of 20 seeds sown individually were used. The polystyrene containers were watered daily at field capacity to maintain moisture. In the greenhouse, temperature during the experiment ranged from 12 to 39 °C, photosynthetic photon flux density (PPFD) ranged from 343 to 1,332.1 µmol $m^{-2}s^{-1}$ and relative humidity ranged from 30.7 to 47.6 %. Seeds were considered germinated when cotyledons were observed. The number of germinated seedlings was recorded daily over a 30-day period.

Mean germination time (*MGT*) was quantified with the following equation (Ranal Marli & García de Santana 2006).

$$MGT = \frac{\sum_{n=1}^{k} n_i t_i}{\sum_{n=1}^{k} n_i}$$

where n_i is the number of seeds germinated at i^{th} time; k is the last day of germination; t_i is the time from the beginning of the experiment until the i^{th} observation time.

Statistical analysis. To compare viability between one-year-old and two-year-old seeds, a Chi-square χ^2 goodness-of-fit test per species was performed. The experimental design was completely randomized with a factorial treatment arrangement. Germination percentage and mean germination time (*MGT*) were compared with a two-way ANOVA by species, with pre-germination treatment and storage time as factors. Prior to analysis, data were normalized by arcsine square root transformation (Sokal & Rohlf 1995) and results were expressed as percentages. Differences between treatments were determined with Tukey's multiple comparison test ($\alpha = 0.05$). All analyses were performed with the GerminaQuant statistical package (Lozano-Isla *et al* 2019) for R software (R Core Team 2022).

Results

Seed viability was more than 50 % in all species except for *Dalea foliolosa* seeds stored for two years, which had the lowest viability (13.3 %) (Table 1). Only *D. foliolosa* and *Paspalum notatum* species absorbed water. *D. foliolosa* absorbed 1 ml in 24 h, while *P. notatum* absorbed 2 ml in 36 h. No water absorption by the rest of the species was observed during the first 48 h.

The two-way ANOVA for pre-germination treatments and storage time for each species showed significant differences among species (Table 2). In addition, the higher germination percentage was obtained by seed scarification with a tweezer pliers. Five of the nine species showed higher germination percentage in seeds stored for two years (Table 3).

Aeschynomene villosa var. *longifolia*, *Crotalaria pumila*, *Desmodium aparines*, *D. tortuosum* and *Zornia reticulata* seeds stored for two years and scarified with a tweezer pliers had higher germination rates than the rest of treatments (Figure 1). *Dalea leporina* and *Desmodium distortun* also had higher germination percentages with the scarification treatment, while storage time had no effect (Figure 1).

The two-way ANOVA revealed statistical differences in mean germination time (*MGT*) by effect of the pre-germination treatments and storage time among species (Table 4), and scarified seeds had shorter germination times. In five species (*Aeschynomene villosa* var. *longifolia, Dalea foliolosa, Desmodium aparines, D. distortun,* and *D. tortuosum*), seed germination was faster with the scarification treatment and two years of storage (Table 5) (Figure 2). *Paspalum notatum* seeds germinated faster with the scarification treatment and one year of storage, while *Zornia reticulata* seeds germinated in a shorter time (*MGT*) with the water bath soaking treatment at 40 °C for 48 hours (IW48) and two years of storage (Figure 2).

Table 1. χ^2 test for seed viability of nine herbaceous plants native to APFFLP collected in 2019 (two years storage) and 2020 (one year storage). Significance value for *P* (< 0.05) in bold type.

Species	Seed viability 2019 (%)	Seed Viability 2020 (%)	*P* value
Aeschynomene villosa var. *longifolia*	76.6	100	0.07827
Crotalaria pumila	90	100	0.4682
Dalea foliolosa	13.3	96	**3.5×10^{-15}**
Dalea leporina	100	96.6	0.8084
Desmodium aparines	86.6	96.6	0.46
Desmodium distortun	86.6	100	0.3266
Desmodium tortuosum	86.6	93.3	0.6174
Paspalum notatum	70	53.3	0.1326
Zornia reticulata	66.6	56.6	0.3676

Table 2. ANOVA results for the germination percentage of pre-germination treatments and storage time of nine native species with potential for restoration of degraded soils of the APFFLP. Significance value for *P* (< 0.05) in bold type.

Species	Pregerminative treatments		Storage time	
	F/df	*P* value	*F*/df	*P* value
Aeschynomene villosa var. *longifolia*	94.9/3	**< 0.0001**	50.9/1	**< 0.0001**
Crotalaria pumila	114.6/3	**< 0.0001**	19.2/1	**< 0.0001**
Dalea foliolosa	4.6/3	**< 0.01**	0.85/1	> 0.3
Dalea leporina	249/3	**< 0.0000**	0.12/1	> 0.7
Desmodium aparines	221/3	**< 0.0000**	2.6/1	> 0.1
Desmodium distortum	80.6/3	**< 0.0000**	4.5/1	**< 0.04**
Desmodium tortuosum	192/3	**< 0.0000**	7.8/1	**< 0.01**
Paspalum notatum	3.4/3	**< 0.03**	4.8/1	**< 0.04**
Zornia reticulata	51.2/3	**< 0.0000**	15.6/1	**< 0.001**

Table 3. Average (± SE) seed germination (%) of nine native herbaceous plants of the APFFLP, with four pre-germinative treatments: C (control), IW24 (immersion in water at 40 °C for 24 hours), IW48 (immersion in water at 40 °C for 48 hours) and P (scarification with tweezer pliers). (1: seeds stored for one year, 2: seeds stored for two years). Different letters denote significant differences, according to the Tukey test ($P < 0.05$) by species.

Treatment	*Aeschynomene villosa* var. *longifolia*	*Crotalaria pumila*	*Dalea foliolosa*	*Dalea leporina*	*Desmodium aparines*	*Desmodium distortum*	*Desmodium tortuosum*	*Paspalun notatum*	*Zornia reticulata*
P/1	46.0 ± 19.8^{b}	50.0 ± 18.7^{b}	19.0 ± 6.5^{a}	93.0 ± 10.3^{a}	87.0 ± 5.7^{b}	87.0 ± 17.8^{a}	71.0 ± 14.7^{b}	2.0 ± 2.7^{b}	45.0 ± 19.0^{b}
P/2	89.0 ± 14.3^{a}	90.0 ± 6.1^{a}	8.0 ± 2.7^{b}	97.0 ± 6.7^{a}	96.0 ± 4.1^{a}	85.0 ± 7.9^{a}	87.0 ± 9.7^{a}	12.0 ± 12.5^{a}	80.0 ± 12.7^{a}
C/1	3.5 ± 1.5^{c}	0.0 ± 0.0^{c}	6.0 ± 8.2^{bc}	5.0 ± 3.5^{bc}	13.0 ± 7.5^{c}	53.0 ± 23.3^{b}	13.0 ± 6.7^{c}	2.0 ± 2.7^{b}	4.0 ± 4.1^{d}
C/2	15.0 ± 11.2^{c}	6.0 ± 4.2^{c}	15.0 ± 11.2^{b}	8.0 ± 5.7^{b}	3.0 ± 6.7^{d}	14.0 ± 2.2^{c}	26.0 ± 9.6^{c}	6.0 ± 5.4^{b}	21.0 ± 13.8^{c}
IW24/1	1.0 ± 1.2^{c}	1.0 ± 1.2^{c}	4.0 ± 4.1^{c}	2.0 ± 4.7^{c}	5.0 ± 7.0^{d}	6.0 ± 4.1^{c}	5.0 ± 3.5^{d}	1.0 ± 2.2^{b}	7.0 ± 6.7^{d}
IW24/2	36.0 ± 5.5^{b}	2.0 ± 2.0^{c}	4.0 ± 4.1^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{d}	6.0 ± 2.2^{c}	6.0 ± 6.5^{d}	0.0 ± 0.0^{b}	10.0 ± 9.3^{d}
IW48/1	1.0 ± 1.2^{c}	4.0 ± 4.1^{c}	7.0 ± 8.3^{b}	1.0 ± 2.3^{c}	13.0 ± 5.7^{c}	6.0 ± 4.1^{c}	8.0 ± 4.5^{d}	4.0 ± 4.1^{b}	5.0 ± 3.5^{d}
IW48/2	37.0 ± 7.5^{b}	4.0 ± 4.1^{c}	5.0 ± 3.5^{c}	0.0 ± 0.0^{c}	11.0 ± 4.0^{c}	11.0 ± 4.2^{c}	6.0 ± 6.5^{d}	10.0 ± 7.9^{ab}	9.0 ± 13.4^{d}

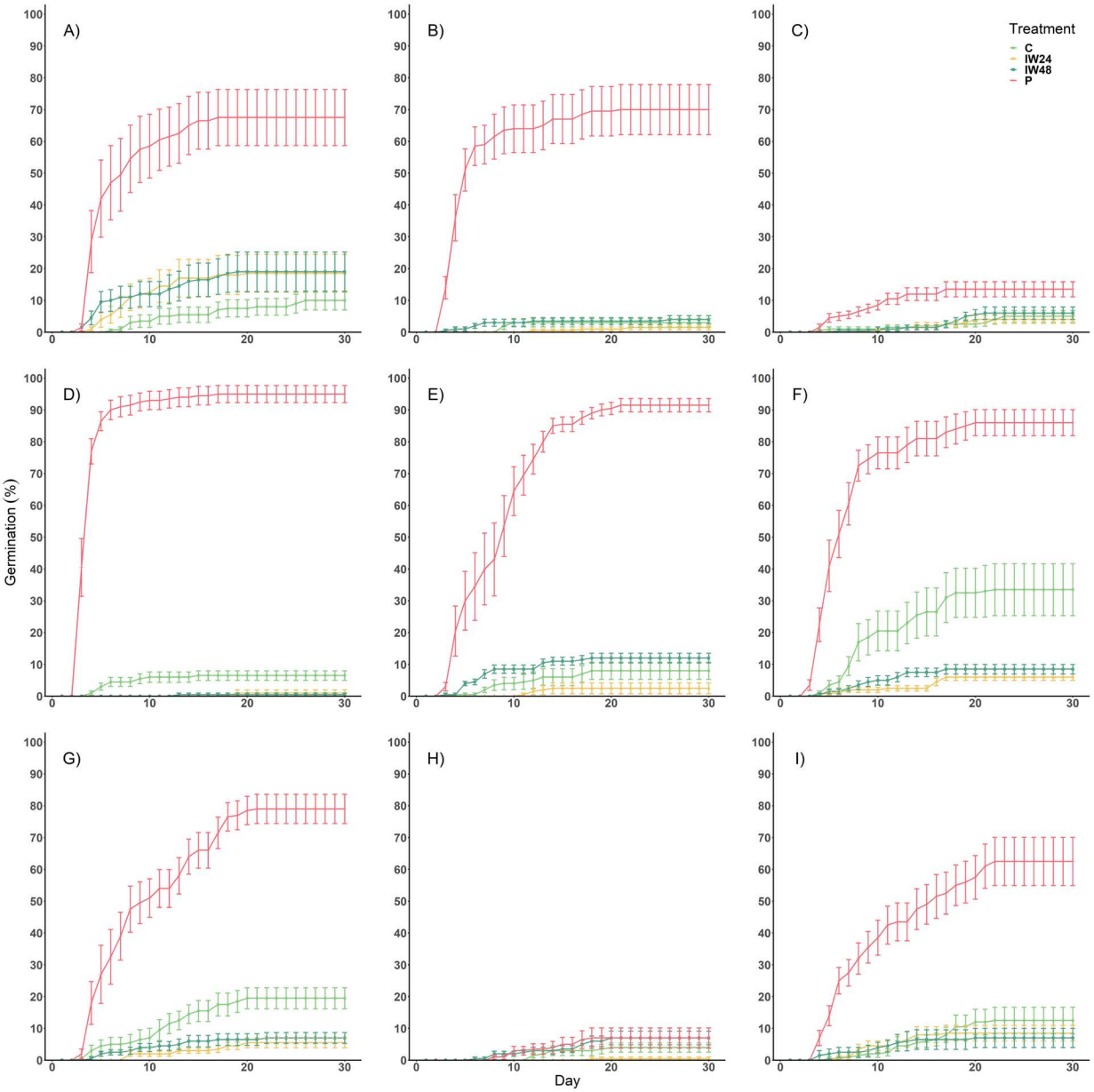

Figure 1. Germination curves of nine native herbaceous A) = *Aeschynomene villosa* var. *longifolia*, B) = *Crotalaria pumila*, C) = *Dalea foliolosa*, D) = *Dalea leporina*, E) = *Desmodium aparines*, F) = *Desmodium distortum*), G) = *Desmodium tortuosum*, H) = *Paspalum notatum* and I) = *Zornia reticulata* of the ANPFFLP; with four pre-germination treatments: C (control), IW24 (immersion in water at 40 °C for 24 hours), IW48 (immersion in water at 40 °C for 48 hours) and P (scarification with a tweezer pliers).

Table 4. ANOVA results for mean germination time (*MGT*) of pre-germination treatments and storage time of nine native species with potential for restoration of degraded soils of the APFFLP. Significance value for P (< 0.05) in bold type.

Species	Pregerminative treatments		Storage time	
	***F*/df**	***P* value**	***F*/df**	***P* value**
Aeschynomene villosa var. *longifolia*	10.8/3	**< 0.0001**	1.04/1	> 0.3
Crotalaria pumila	12.9/3	**< 0.0001**	0.41/1	> 0.5
Dalea foliolosa	7.8/3	**< 0.001**	4.9/1	**< 0.04**
Dalea leporina	19.8/3	**< 0.0000**	0.16/1	> 0.6
Desmodium aparines	1.1/3	> 0.3	15.2/1	**< 0.001**
Desmodium distortum	6.9/3	**< 0.001**	2.03/1	> 0.1
Desmodium tortuosum	1.4/3	> 0.4	0.6/1	> 0.2
Paspalum notatum	1.8/3	> 0.1	0.02/1	> 0.8
Zornia reticulata	0.6/3	> 0.5	0.6/1	> 0.6

Table 5. Average (± SE) mean germination time (*MGT*) of seeds of nine native herbaceous plants from the APFFLP with four pre-germinative treatments: C (control), IW24 (immersion in water at 40 °C for 24 hours), IW48 (immersion in water at 40 °C for 48 hours) and P (scarification with tweezer pliers). (1: seeds stored for one year, 2: seeds stored for two years). Different letters denote significant differences, according to the Tukey test (P < 0.05) by species.

Treatment	***Aeschynomene villosa* var. *longifolia***	***Crotalaria pumila***	***Dalea foliolosa***	***Dalea leporina***	***Desmodium aparines***	***Desmodium distortum***	***Desmodium tortuosum***	***Paspalun notatum***	***Zornia reticulata***
P/1	9.0 ± 2.3^{ab}	5.5 ± 1.0^{a}	10.7 ± 1.5^{b}	4.0 ± 0.6^{a}	10.9 ± 0.8^{b}	7.9 ± 1.4^{ab}	11.5 ± 0.7^{ab}	8.0 ± 1.0^{a}	10.5 ± 1.9^{ab}
P/2	5.2 ± 0.7^{a}	5.6 ± 0.6^{a}	4.7 ± 0.5^{a}	4.0 ± 0.8^{a}	6.6 ± 1.1^{a}	5.8 ± 1.3^{a}	7.2 ± 9.7^{a}	14.6 ± 1.8^{ab}	10.0 ± 1.9^{ab}
C/1	11.3 ± 1.8^{b}	0.0 ± 0.0	15.0 ± 7.0^{bc}	4.6 ± 1.5^{a}	12.0 ± 0.7^{b}	11.0 ± 1.9^{b}	13.0 ± 6.7^{b}	19.5 ± 1.7^{b}	8.0 ± 3.1^{a}
C/2	17.4 ± 6.2^{c}	9.3 ± 4.6^{b}	17.2 ± 6.0^{c}	9.0 ± 4.3^{b}	6.6 ± 6.7^{a}	11.1 ± 1.8^{b}	10.5 ± 2.2^{ab}	13.0 ± 1.0^{ab}	16.0 ± 1.6^{b}
IW24/1	20.0 ± 1.0^{c}	17.0 ± 7.1^{c}	16.1 ± 6.2^{c}	18.5 ± 4.7^{c}	12.5 ± 0.7^{b}	13.2 ± 3.2^{b}	11.0 ± 3.5^{ab}	18.0 ± 1.2^{b}	10.8 ± 4.3^{ab}
IW24/2	8.7 ± 1.5^{ab}	17.0 ± 6.0^{c}	13.8 ± 3.0^{bc}	0.0 ± 0.0	0.0 ± 0.0	11.6 ± 5.5^{b}	14.1 ± 6.5^{b}	0.0 ± 0.0	10.4 ± 1.0^{ab}
IW48/1	7.0 ± 1.0^{ab}	9.1 ± 0.5^{b}	19.0 ± 0.5^{c}	13 ± 2.3^{bc}	8.7 ± 3.6^{ab}	11.2 ± 2.8^{b}	10.0 ± 5.6^{ab}	11.0 ± 1.1^{ab}	12.0 ± 6.2^{b}
IW48/2	8.6 ± 1.5^{ab}	7.8 ± 3.6^{ab}	13.0 ± 3.0^{bc}	0.0 ± 0.0	6.7 ± 1.6^{a}	9.1 ± 1.7^{b}	13.0 ± 6.5^{b}	13.0 ± 4.8^{ab}	7.2 ± 4.6^{a}

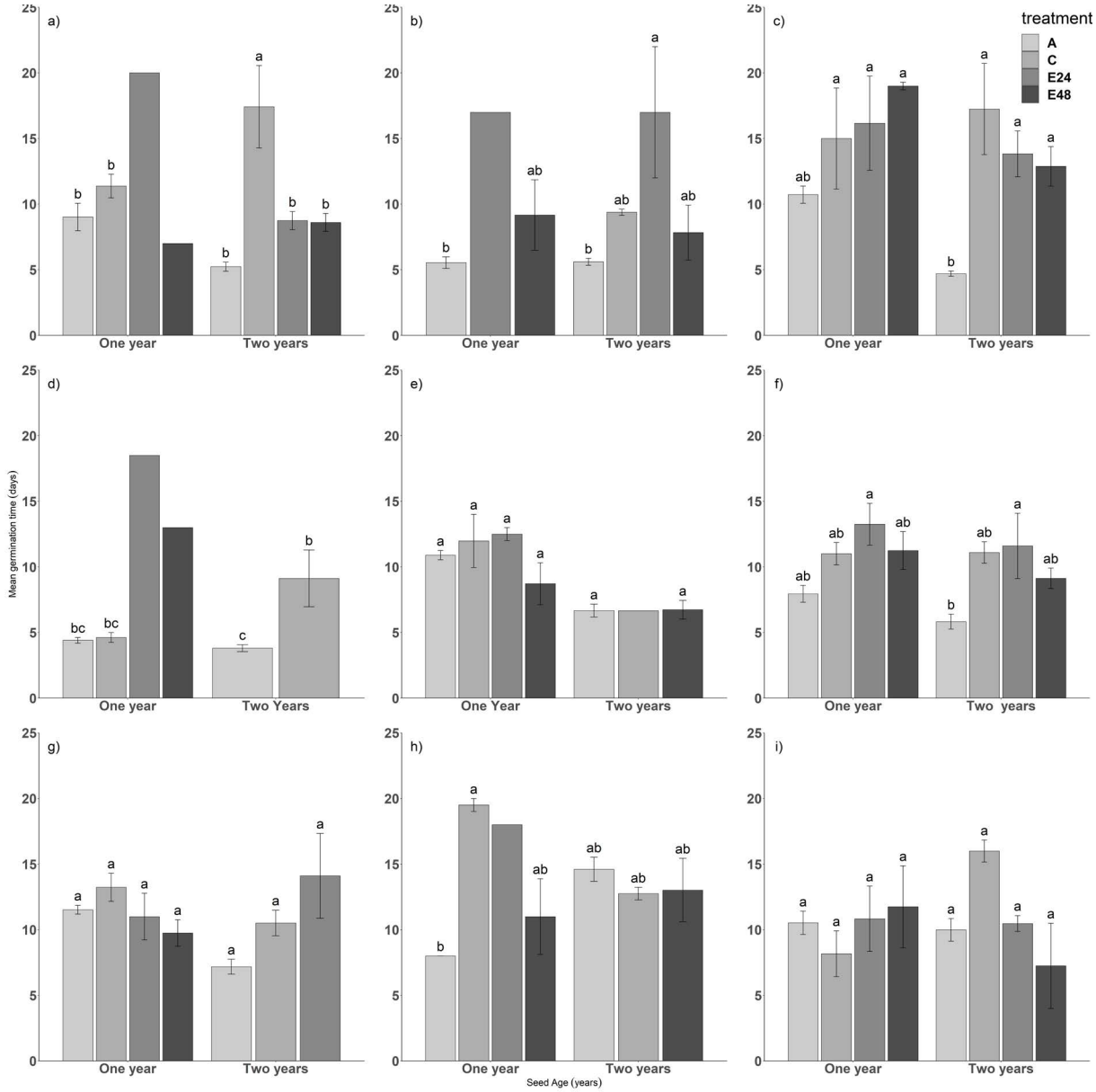

Figure 2. Mean (± SE) mean germination time (*MGT*) for nine native herbaceous A) = *Aeschynomene villosa* var. *longifolia*, B) = *Crotalaria pumila*, C) = *Dalea foliolosa*, D) = *Dalea leporina*, E) = *Desmodium aparines*, F) = *Desmodium distortum*, G) = *Desmodium tortuosum*, H) = *Paspalum notatum* and I) = *Zornia reticulata*) of the ANPFFLP; with four pre-germination treatments: C (control), IW24 (immersion in water at 40 °C for 24 hours), IW48 (immersion in water at 40 °C for 48 hours) and P (scarification with a tweezer pliers). Different letters denote significant differences, according to the Tukey test ($P < 0.05$).

Discussion

Frequent fires in the APFFLP have caused soils to become devoid of plants and, therefore, subject to erosion, loss of fertility, and biodiversity, among others (SEMARNAT 2000). Native herbaceous plants can be used to protect the soil from erosion. About 70% of seed-bearing plants show dormancy (Willis *et al*. 2014), a limitation for seedling establishment in degraded sites. Germination mechanisms of native plants provide basic information of the regeneration processes of plant communities with ecological restoration activities (Sales *et al*. 2013). However, seed dormancy of

native plants could represent an obstacle for their establishment and a limitation for the seeds to be used in restoration plans and projects. Clarifying the mechanisms that lead to the elimination of seed dormancy could enhance success of actions for ecological restoration (Jiménez-Alfaro *et al*. 2016, Baskin & Baskin 2020).

Establishment of native plants is needed for restoration of degraded soils (Meli *et al*. 2014). Seed viability impacts the potential of seeds to germinate and their ability to colonize new spaces (Mancipe-Murillo *et al*. 2018). Several studies include Fabaceae and Poaceae for restoration projects. For example, Citadini-Zanette *et al*. (2017) compared the establishment of seedlings of three native Fabaceae trees (*Mimosa scabrella* Benth., *Schizolobium parahyba* (Vell.) Blake, and *Bastardiopsis densiflora* (Hook. & Arn.) Hassl.) against exotic species [(*Eucalyptus saligna* Sm., *E. viminalis* Labill., *E. citriodora* Hook., *Grevillea hilliana* F.Muell., *Hovenia dulcis* Thunb., *Melia azedarach* L., *Pinus elliottii* Engelm., *P. taeda* L., and *Syzygium cumini* (L.)]. These authors found that *M. scabrella* displayed clear evidence of restoration in progress. Godinez-Álvarez & Flores-Martínez (1999) found that sandpaper scarification of *Chloris gayana* Kunth and *Cenchrus ciliaris* L. seeds (identified as useful species for restoration of the coast of Guerrero, Mexico) showed germination percentages between 50 and 60 %.

In this study, seeds of seven species (*Aechynomene villosa* var. *longifolia*, *Crotalaria pumila*, *Dalea foliolosa*, *D. leporina*, *Desmodium aparines*, *D. distortum*, and *D. tortuosum*) (Fabaceae) of the nine studied showed a high percentage of viability (> 90 %) after 12 months of storage. Seed viability may vary between years of collection due to environmental conditions affecting the parent plant (Elizalde *et al*. 2017); however, in this study, only *D. foliolosa* showed differences in viability with respect to the year of collection. For the two-year-old seeds of *Dalea foliolosa*, germination was 8 %; this is related to low seed viability (13.3 %). However, for one-year-old seeds, viability was 96 %, but only 19 % germinated, suggesting that the embryos were too immature for germination. The loss of seed viability may be due to factors such as maternal effects, pre-dispersal, seed predation, and environmental stress during development and maturation, etc. (Baskin & Baskin 2004). However, seed damage by beetles (Bruchidae) is a common cause of decreased seed viability in Fabaceae (El Atta 1993, Tomaz *et al*. 2007, Parra-Gil *et al*. 2020). All these factors may have influenced the low viability and germination of the *D. foliolosa* seeds collected in 2019. Viability and germination of fresh seeds remain to be tested.

We found that all nine species exhibited dormancy. Seeds that do not imbibe water are considered to have physical dormancy (Baskin *et al*. 2000). The mechanism that inhibits imbibition or absorption of water in seeds having physical dormancy is a hard seedcoat that prevents germination (Baskin & Baskin 2004). This impermeability of the coat is caused by one or more palisade layers of lignified malphigian cells (called macrosclereids) strongly packed together and permeated with water-repellant chemicals (Baskin 2003). *Zornia reticulata* also imbibed water so it did not present physical dormancy. However, scarification with tweezer pliers and storage for two years significantly increased germination; hence, they also had physiological dormancy. In seeds of *Z. diphylla* it has been found that embryos may mature six months after collection (Singh 1976).

For *Paspalum notatum*, even though it was soaked in water, no germination was recorded, indicating physiological dormancy. Seed dormancy has also been found in other *Paspalum* spp., which have hard covers (palea and lemma) that confer mechanical resistance and prevent germination (Fulbright & Flenniken 1988).

Seed dormancy is a selective mechanism ensuring that germination occurs under suitable environmental conditions (Jurado & Flores 2005). Several studies refer to physical dormancy in seeds of the Fabaceae family (Jayasuriya *et al*. 2013, Erickson *et al*. 2016), due to the presence of hard seedcoats that impede water absorption and gas exchange (Baskin & Baskin 2004, Sánchez *et al*. 2019). Our results revealed that five (*Aeschynomene villosa* var. *longifolia*, *Crotalaria pumila*, *Desmodium aparines*, *D. tortuosum* and, *Zornia reticulata*) of the nine species studied (all Fabaceae) reached germination higher than 80 % when they were scarified, which proved to be the most effective method for breaking dormancy. We also found that *Dalea foliolosa* (Fabaceae) and *Paspalum notatum* (Poaceae) seeds recorded the lowest germination rate, despite being the only ones that imbibed water, suggesting that embryos were too immature for germination (physiological dormancy).

Among the treatments to eliminate dormancy in hard-coated seeds is immersion in hot water between 40-100 °C for a variable period, which can increase germination through the influx of water and oxygen (Tadros *et al*. 2011). In

our study, water immersion treatments at 40 °C for 24 (IW24) and 48 hours (IW48) in seeds with one and two years of storage had no effect on breaking seed dormancy of the species studied.

In degraded areas, the rapid establishment of plants based on the use of seeds is a useful and crucial method to grow ground cover and decrease erosion (Larson & Funk 2016). Successful restoration requires seeds with high germination speed efficiency (Godinez-Álvarez & Flores-Martínez 1999, Kildisheva *et al* 2020). Mean germination time (*MGT*) is an indicator of the time it takes for seeds to germinate. It is considered that species that germinate faster are more successful in establishing in a community (Sánchez *et al*. 2019). We found that the seeds of *Aeschynomene villosa* var. *longifolia, Dalea foliolosa*, *Desmodium aparines*, *D. distortum* and *D. tortuosum* treated by scarification with a tweezer pliers and two years of storage showed faster germination speed, although the seeds of *Crotalaria pumila* and *Dalea leporina* with one or two years of storage also had faster germination speed. These species could therefore be used for projects to restore degraded soils of La Primavera Forest, as this condition is an advantage for seedling establishment to obtain rapid cover on degraded soils (Pedrini *et al*. 2019).

Other studies have shown the importance of scarification to break dormancy and enhance germination percentage in Fabaceae and Poaceae (Uzun & Aydin 2004, Romo-Campos *et al*. 2009, Delgado *et al*. 2015). West & Marousky (1989), in *P. notatum*, higher germination percentage in aged seeds after eliminating the lemma. Also, immersion in hot water and mechanical scarification can make impermeable seeds of many Poaceae species permeable, including *Paspalum notatum* (Baskin & Baskin 2004). In this study, none of the treatments proved to be effective in achieving high germination percentage in *P. notatum* even though 70 % of the seeds were viable, probably due the hardness of the seeds and the floral structures such as the lemma and the palea that prevent the passage of water to the embryo (Baskin & Baskin 2004). For *Paspalum notatum*, more studies are needed to identify treatments that could break seed dormancy and thus increase seed germination. Our findings provide basic information on the germination of native species that could help restore disturbed sites in the La Primavera Flora and Fauna Protection Area.

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