

Does seed aggrupation and substrate type affect the germination on three native species of Durango, Mexico?

¿Afecta la agrupación de semillas y el tipo de sustrato la germinación de tres especies nativas de Durango, México?

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Abstract. The chemical and physical properties of the substrate produce positive or negative effects on the germination of seeds, and different pre-germination treatments are applied to obtain better results. However, the use of soil as a substrate where the species grows is usually not evaluated or used as a treatment. The objective of the present study was to evaluate the effect of the native substrate [two types of substrate where the woody species grow (clay and sand)] on the germination of *Acacia farnesiana*, *Larrea tridentata* and *Prosopis laevigata*. In addition, two groups were compared in seeds (grouped and not grouped) of the protected area "Parque Estatal Cañón de Fernández". The results showed that the seed grouping reduced germination in the evaluated species, while the germination rate was only affected in *Prosopis laevigata* in the evaluated substrates.

Keywords: Canyon de Fernández; Native vegetation; Treatments and seed grouping.

Resumen. Las propiedades químicas y físicas del sustrato producen efectos positivos o negativos en la germinación de semillas por lo que se aplican diferentes tratamientos pre-germinativos para obtener mejores resultados. Sin embargo, el uso del suelo como sustrato donde crecen las especies, por lo general no se evalúa ni se utiliza como tratamiento. En el presente estudio el objetivo fue evaluar el efecto del sustrato [dos tipos de sustratos nativos donde crecen las especies leñosas (arcilla y arena)] en la germinación de *Acacia farnesiana*, *Larrea tridentata* y *Prosopis laevigata*. Además se compararon dos condiciones de agrupamientos de las semillas (agrupadas y no agrupadas) procedentes del área protegida "Parque Estatal Cañón de Fernández". Los resultados mostraron que el agrupamiento de semillas disminuyó la germinación en las especies evaluadas, mientras que la velocidad de germinación solo fue afectada en *Prosopis laevigata* en los sustratos evaluados.

Palabras clave: Cañón de Fernández; Vegetación nativa; Tratamientos y agrupamiento de semillas.

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INTRODUCTION

In addition to internal conditions, seeds require external factors (e.g., temperature, humidity and substrate conditions) to germinate. The physicochemical characteristics of the substrate may promote or inhibit germination. Common commercial substrates, and mixtures of these, have been frequently used in attempting to obtain higher productivity and lower seedling loss after germination (Aparicio et al., 1999). This has been done without considering that the substrates where the plants develop naturally can be more economical and suitable. In addition, natural substrates might contain important fungi for the germination of the seeds, and subsequent plant growth (Molina et al., 2005). In this sense, *Acacia farnesiana* (L.) Willd., *Larrea tridentata* (Sessé and Moc. Ex DC.) Coville and *Prosopis laevigata* (Humb. & Bonpl. Ex Willd.) M.C. Johnst. have been found to be distributed in soils of clay and sandy textures (Biodesert, (2003).

It is necessary to consider the evolutionary aspects of the seeds that can promote or inhibit germination. For example, high densities of clustered seeds may result in low germination percentages as a response to population regulation (Callaghan, 1996; McMurray et al., 1997; Lortie & Turkington 2002) or for reducing competition among sow seedlings (Biedrzycki et al., 2010). For species that have a cluster dispersion (clump dispersal) the competition among seeds tends to limit germination at higher densities. This will contribute to reduce competition between seedlings (Cheplick 1992, 1993; McMurray et al., 1997; Murray, 1998; Lortie & Turkington, 2002; Grundy et al., 2003; Dyer, 2004; Flores & Jurado, 2009). There is also evidence of species that require the presence of seed groups to increase seedling survival and establishment (Muro et al., 2013). The characteristics of the type of dispersion and seed grouping mentioned above are mechanisms that occur in seeds of *Acacia farnesiana*, *Larrea tridentata* and *Prosopis laevigata*. In this sense, the seeds, establishment and survival of native species are contingent on the quality of the site, so that in these areas it is possible to find seeds suitable for the regeneration of the "Canyon of Fernandez" ecosystem, where agricultural and livestock activities are conducted.

Considering that the species are native, present density high, and low dispersion of fruits, they are restoring the deterioration in areas where they are distributed (Kuschik, 2004). No studies on their germination have been carried out, evaluating the effect of the seed grouping and the type of substrate. The present study was carried out starting from the central hypothesis that the separated seeds will germinate in a greater percentage independently of the type of substrate where they are distributed. This will contribute to a greater plant reproduction which will contribute to the ecological restoration of disturbed areas.

MATERIALS AND METHODS

Collection of seeds and obtaining the substrate. During 2009, fruits of *A. farnesiana* trees, *L. tridentata* and *P. laevigata* shrubs were collected, distributed in the microphyllous and mesquite desert scrub of the Fernandez Canyon (Muro et al., 2012). Seeds of the species were manually extracted from pods (*P. laevigata* and *A. farnesiana*) and mature pericarps (*L. tridentata*) (Sánchez & Ramírez, 2006). They were dried at room temperature (Ríos et al., 2010). Each seed was revised to discard those with anomalies (Hernández et al., 2001) and insect damage (Sánchez & Ramírez, 2006). The two types of soil were collected where the species develop in the study area (clay soil and sand), and a chemical and physical analysis was performed on the soil (Valderrama et al., 2005; Muro, 2012).

Treatment of seeds. The seeds of *A. farnesiana* and *P. laevigata* were scarified by immersion in 100% H₂SO₄ for five minutes (Rivas et al., 2005). The pericarp of *L. tridentata* seeds was manually removed (CONABIO, 2011a). The seeds were planted in black bags for nursery of 17 x 17 cm, caliber 400 with capacity of 0.95 liters, and every third day were watered to maintain the humidity. Each treatment consisted of five replicates with 10 seeds each, giving a total of 50 seeds per treatment (3 species x 2 types of scarification x 2 soil types x 5 replicates x 10 seeds/replicate = 600 seeds of all species) and 200 seeds per species in both treatments. A completely randomized factorial design was applied (Larenas & De Viana, 2005).

Evaluation of germination. Germination evaluation methods were grouped and non-grouped seeds. The first consisted of placing groups of 10 seeds per experimental unit (bag), and the second in forming two rows of five seeds within each bag with a separation of 2 centimeters between rows and 5 centimeters within each line. Germination was compared through the interaction species * treatments as follows: 1) grouped in clay, 2) non-grouped in clay, 3) grouped in sand and 4) non-grouped in sand, using ANOVA (D'Aubeterre et al., 2002).

Statistical analysis. The Tukey test was applied to make multiple comparisons between the means of treatments by species, after opening the interaction (García et al., 2007; Saucedo et al., 2009). The germination rate (t50) was then calculated as the time elapsed in days from seed sowing until reaching 50% germination (Díaz, 1993; Rossini et al., 2006). The data were transformed with the square root arcsine to adjust for normality (Sokal & Rohlf, 1995). The germination records were performed daily for a period of 32 days (Sánchez & Ramírez, 2006) during which the minimum temperature was 22 °C ± 0.9 E.E., and the maximum of 30.6 °C ± 0.7 E.E. A seed was considered germinated after radicle emergence

(Hernández et al., 2001). The laboratory works were carried out in the greenhouse of the Facultad de Ciencias Biológicas of the UJED.

RESULTS

Percentage of germination. Treatments by species. The treatments of clustered seeds (clay and sand) showed no difference in germination. The treatment of seeds not grouped in clay soil showed a significant ($F = 5.848$; $P = 0.016$) difference within germination between species. The highest percentage of germination occurred in *L. tridentata* ($58\% \pm 0.25$ E.E.) and the lowest in *A. farnesiana* ($30\% \pm 0.30$ E.E.). This response was presented in the treatment of non-grouped sand ($F = 9,551$; $P = 0.003$). *Larrea tridentata* showed the highest germination ($38\% \pm 0.58$ EE), and *A. farnesiana* the lowest germination ($6\% \pm 0.26$ EE) among species (Table 1).

With the exception of *P. laevigata* in the treatment of seeds grouped in clay soil ($F = 4.131$, $P = 0.02$), the seeds of *A. farnesiana* and *L. tridentata* showed no differences in germination in the remaining treatments (Table 2).

Cumulative germination by species since the beginning of the evaluation. The treatment of Grouped in clay (GIC) presented germination percentages greater than 50% in the three species, as well as the treatment of Non-clustered Clay (NCC) in *Larrea tridentata* (58%). The rest of the treatments in the three species responded in a similar way showing a

minimum germination value of 28% in *P. laevigata* in the treatment of Non-clustered Sand (NCS), and 48% germination in Grouped in Clay (GC) for *L. tridentata* (Fig. 1).

Velocity of germination (t50). Treatments by species. The germination speed was not affected by treatments on any species: all species showed a similar response within each treatment (Table 3).

The seeds of *P. laevigata* germinated at higher rates ($F = 4,131$; $P < 0.05$) when the seeds were non-clustered on sand. The longest time to germinate was shown on grouped seeds in clay soil. *A. farnesiana* and *L. tridentata* showed similarity in speed in all treatments (Table 4).

Soil analysis. Average temperatures (27.56 ± 1.24 °C), pH (5.82 ± 0.67) and organic matter concentrations (4.67 ± 0.072) were recorded in spring 2009. Average particle size in sand was 0.415 mm (Table 5).

DISCUSSION

Germination. An adequate pre-germination treatment will depend on the characteristics of the seed to be reproduced. The evaluated experiments showed that the treatment interaction * species produces different percentages of germination, independently of the applied scarification process. The germinative variations were basically presented in the treatment

Table 1. Percentage of germination obtained for each treatment by species.

Tabla 1. Porcentaje de germinación obtenido para cada tratamiento en las distintas especies.

Treatments	<i>Acacia farnesiana</i>	<i>Prosopis laevigata</i>	<i>Larrea tridentata</i>
Grouped in clay	52 ± 0.44 a	62 ± 0.13 a	68 ± 0.25 a
Non clustered clay	30 ± 0.30 a*	40 ± 0.47 b*	58 ± 0.25 c*
Grouped sand	34 ± 0.26 a	32 ± 0.33 a	48 ± 0.39 a
Non clustered sand	6 ± 0.26 a**	28 ± 0.39 b**	38 ± 0.58 c**

The different seed groupings by type of substrate for the three species are shown evaluating the effect of the clustering on germination. Percentage ± standard error. Different letters within each row indicate significant differences between treatments among the different species ($P < 0.05$). * Significant difference. ** Highly significant difference.

Table 2. Percentage of germination obtained for each species by treatment.

Tabla 2. Porcentaje de germinación obtenido por cada especie en los diferentes tratamientos.

Species	Grouped in clay	Non clustered clay	Grouped sand	Non clustered sand
<i>Acacia farnesiana</i>	52 ± 0,44 a	30 ± 0,3 a	34 ± 0,3 a	6 ± 0,3 a
<i>Prosopis laevigata</i>	62 ± 0,13 a*	40 ± 0,47 b	32 ± 0,33 b	28 ± 0,39 b
<i>Larrea tridentata</i>	68 ± 0,25 a	58 ± 0,25 a	48 ± 0,39 a	38 ± 0,58 a

The species grouped by type of substrate are shown evaluating the effect of the substrate for germination. Percentage ± standard error. Different letters within each row indicate significant differences among treatments within each species ($P < 0.05$). *Significant difference.

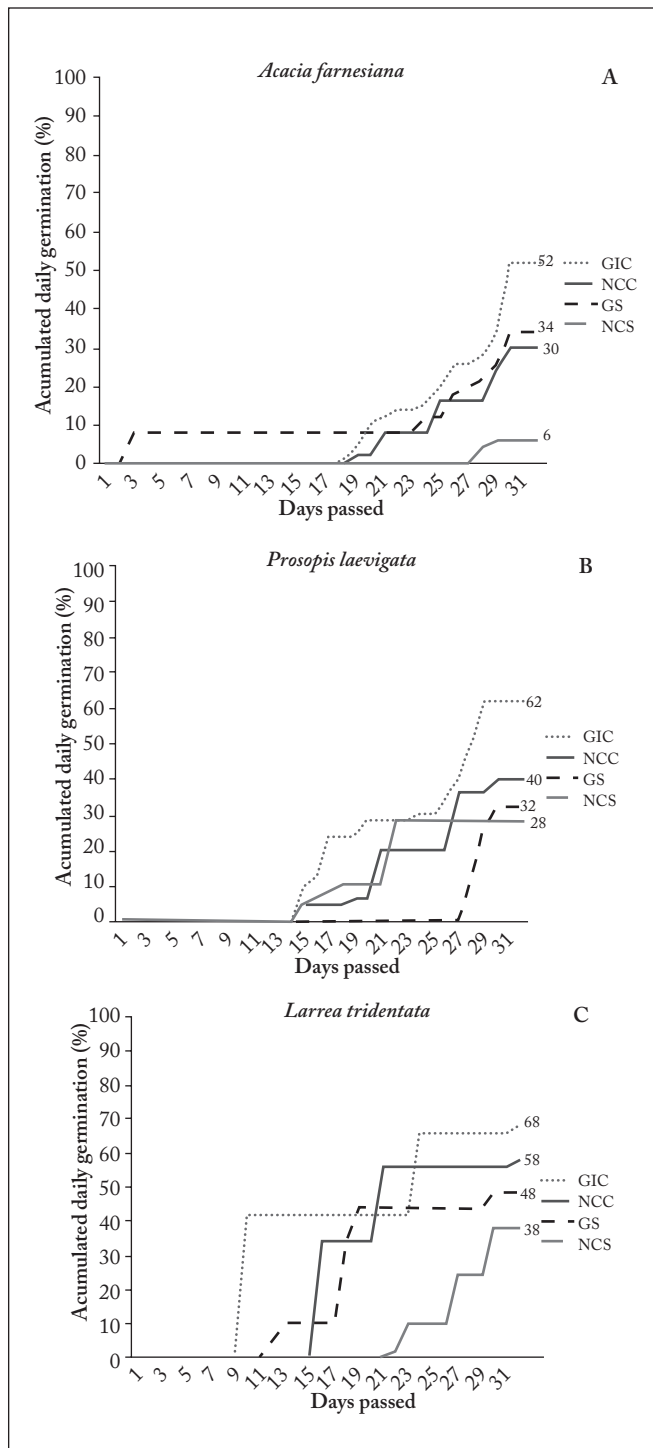


Fig. 1 Behavior of accumulated daily germination in the three species (A) *Acacia farnesiana* (B) *Prosopis laevigata* and (C) *Larrea tridentata*. Where GIC is Group in clay, NCC is Non Clustered Clay, GS is Grouped sand and NCS is Non Clustered Sand.

Fig. 1. Respuesta de la germinación diaria acumulativa en las tres especies (A) *Acacia farnesiana* (B) *Prosopis laevigata* and (C) *Larrea tridentata*. GIC es agrupamiento en arcilla, NCC es no agrupación de semillas en arcilla, GS indica semillas agrupadas en arena, y NCS indica semillas no agrupadas en arena.

“non-grouped seeds for clay and sand substrates” ($F = 5.848$; $P = 0.016$). According to Godínez & Flores (1999) specific pre-germination treatments such as mechanics and chemistry are indicated for seeds that present dormancy, curious teguments or low germinability because they require endozoic ingestion to increase their germinative vigor (Burkat, 1952; Bouma et al., 1979; González, 2002; Domínguez et al., 2006).

Authors such as Offiong et al. (2010) (*Tectona grandis*); Al-Sherif (2007) (*Prosopis farcata*); Peláez et al. (1992) (*Prosopis caldenia* 91% germination); Dehghan et al. (2003) (*Lupinus dif-fusa*); D'Aubeterre (2002) (3 species of *Prosopis*); Ortega et al. (2002) (*Prosopis ferox*); Hernández et al. (2001) (*Enterolo-bium*); Vilela & Ravetta (2001) (5 species of *Prosopis*); Padma et al. (1995); Masamba (1994) (*Acacia* sp), have used chemical scarification with sulfuric acid to increase the percentages of germination mainly in seeds of the family Fabaceae and Lamiaceae. The effect of sulfuric acid is the softening of the cover by oxidation, increasing the permeability of air and water through the seed envelope (Hartmann & Kester, 1980).

However, other authors consider that chemical scarification will give similar results because the seeds have coarse integuments that provides them with a degree of imposed latency which decreases when applying sulfuric acid because it allows the entrance of water and exchange of gases (Harper, 1977, Padma et al., 1995). On the other hand, Prokopiuk & Chifa (2000) mention that the absorption of water through the tegumentary cover at a temperature of 30 °C causes a continuous germination, whereas between 20 and 40 °C the effect of the temperature directly affects the regulation of the amount and rate of water absorption, causing lethargy in germination.

According to Prokopiuk & Chifa (2000) we can consider that the low percentages of germination were not due to temperature, since it was constant during the evaluation, but rather to the specific germination requirements of each species Godínez & Flores (1999). Another explanation for the low germination specifically in the sand substrate is the loss of moisture from the soil (Martínez et al., 1997) due to sand. This is because of its high permeability, physical properties that retain moisture for a shorter time (Segura et al., 2008). It can be also due to problems in the internal development of seeds (Harper, 1977). The results in percentage of germination of the present study, applying pure sulfuric acid for 10 minutes generate a germination response similar to that obtained by the authors mentioned above and which varied between 60 and 100% depending on the species.

Velocity of germination (t_{50}). According to Taylor et al. (1999), the germination velocity can be increased directly with the increase in temperature since the seeds respond to temporal fluctuations. Possibly the speed was not affected because the seeds require higher temperatures to accelerate germination as mentioned by Enríquez et al. (2004). These authors determine that a temperature of up to 35 °C can produce effects on the germination speed on seeds of the same species, and among trees of the same site.

Table 3. Velocity of germination obtained for each treatment by species.**Tabla 3.** Velocidad de germinación obtenida para cada tratamiento en las diferentes especies.

Treatments	<i>Acacia farnesiana</i>	<i>Prosopis laevigata</i>	<i>Larrea tridentata</i>
Grouped in clay	23,8 ± 11,66 a	26,2 ± 3,12 a	18,4 ± 6,72 a
Non clustered clay	12,4 ± 14,88 a	17 ± 13,67 a	19 ± 2,40 a
Grouped sand	16,8 ± 13,83 a	6,2 ± 12,15 a	13,4 ± 11,48 a
Non clustered sand	4,6 ± 7,81 a	4,4 ± 8,62 a	12 ± 14,40 a

The mean ± standard error for each treatment evaluated is shown. Different letters within each row indicate significant differences among species in the same treatment (P<0.05).

Table 4. Velocity of germination obtained for each species by treatment.**Tabla 4.** Velocidad de germinación obtenida en cada especie en los diferentes tratamientos.

Species	Grouped in clay	Non clustered clay	Grouped sand	Non clustered sand
<i>Acacia farnesiana</i>	23,8 ± 11,6 a	12,4 ± 14,88 a	16,8 ± 13,83 a	4,6 ± 7,81a
<i>Prosopis laevigata</i>	26,2 ± 3,12 a*	17 ± 13,67 b*	6,2 ± 12,15 c*	4,4 ± 8,62 d*
<i>Larrea tridentata</i>	18,4 ± 6,72 a	19 ± 2,40 a	13,4 ± 11,48 a	12 ± 14,40 a

The mean ± standard error obtained by species in each treatment is shown. Different letters within each row indicate significant differences among treatments in the different species (P<0.05). *Significant difference.

Table 5. Results of the chemical and physical analysis performed on the soils where the species are distributed.**Tabla 5.** Resultados de análisis físico-químicos en los suelos donde están distribuidas las especies.

Clay soil	Variable	Sand
-	Granulometry (mm)	0.415 ± 0.301
27.56 ± 1.24 ▼	Temperature (°C)	38.75 ± 2.15 ▲
5.82 ± 0.067 ▼	pH	8.37 ± 0.021 ▲
0	Soil moisture (%)	0
4.67 ± 0.072	Organic material (%)	1.40 ± 0.041
37.01 ± 0.385 ▼	Sand (%)	60.48 ± 0.032 ▲
42.89 ± 0.074	Limo (%)	26.75 ± 0.211
20.10 ± 0.149	Clay (%)	12.77 ± 0.008
0.27 ± 0.012 ▼	Total de N (%)	7.03 ± 0.051 ▲
32.11 ± 1.121	P (ppm)	23.49 ± 0.008
0.27 ± 0.008	K (meq/100 g)	0.19 ± 1.004
0.24 ± 0.034	Na (meq/100 g)	18.14 ± 0.411
23.27 ± 0.431	Ca (meq/100 g)	9.28 ± 0.251
3.01 ± 0.435	Mg (meq/100 g)	1.75 ± 0.491
	Exchange capacity	
21.23 ± 0.678 ▲	Cationic (meq/100 g)	8.94 ± 0.571 ▼

The variables of temperature, pH, sand (%), nitrogen (%) and cation exchange capacity determined the differences between the two types of substrate where the species were distributed. The triangles indicate higher percentage (▲) or lower percentage (▼).

Density germination. Seed grouping is a poorly studied germination strategy. According to Flores & Jurado (2009) it is a dynamic process that regulates populations, increasing the percentage of germination when the seeds are grouped. In this sense, and according to McMurray et al. (1997), germination on seed treatments grouped in both clay and sand was higher than that of non-clustered seeds. A high seed density occurs in fruits that require endozoic ingestion such as *A. farnesiana* and *P. laevigata* when they are thrown in the animal feces. This process increases the viability of seeds (Rodríguez et al., 2005). In this respect, we can say that although 60% of germination was obtained, the rest of the seeds have a high probability of being viable according to Traveset & Riera (2005). Possibly if the germination evaluation time had been increased, the percentages might have reached 90% CONABIO (2011).

In cacti of arid environments, it was found that the increase of seed density may or may not affect germinability as in *Isolatocereus dumortieri*, where seed grouping diminished germination, whereas in *Myrtilocactus geometrizans*, seed grouping produces a constant germinability effect (Flores & Jurado, 2009). Seed grouping produced results that may be mistaken because of the seeds intrinsic effects (latency) or the substrates own effects (Flores et al., 2006 & 2008).

Effect of the substrate. Ecologically, plants produced in native substrata have a higher tolerance to the environmental (Cox & Moore, 1994) and physiological stresses (Levitt, 1980), unlike those reproduced in inert or composite substrates (Muñoz, 2007). The germination response that

was presented in “Grouped in the clay” treatment may be related to that reported by Cox & Moore (1994), Levitt (1980) and Muñoz (2007): the species responded favorably to the use of native soil (grouped in clay) with germination percentages greater than 50%. This is because native substrates provide them with the necessary nutrients making the species resistant to extreme drought and low temperature events (Van den Driessche, 1963). It is important to note that regardless of the low percentages of germination obtained in this study, the plants have the appropriate conditions for their development and establishment, allowing their use in reforestation programs and restoration of degraded ecosystems.

It has been determined that high percentages of germination are the result of using soils rich in organic matter (García et al., 2011) or nitrogen (Andrade-Rodríguez et al., 2008), which may explain the similar germination percentages obtained in this study in Sandy soil with clustered seeds. Unfortunately the use of substrates for germination has focused on finding the optimal soil to obtain high germination percentages with economical interest (Vega, 1986), no matter how expensive it might be (Sánchez-Salas et al., 2012; Aparicio et al., 1999). In addition to issues of the experimental part, it is necessary to consider the basic technological conditions that can be useful to the inhabitants. For example, they might directly benefit from the services that the native species (e.g., *A. farnesiana*, *P. laevigata* and *L. tridentata*) can provide: wood, charcoal, handicraft, food and even medicines (Rivas et al., 2005). On the other hand, the application of accessible and economical techniques are a viable alternative that not only benefits the inhabitants but also contributes directly to the conservation of the local populations by reducing the pressure for extraction of the renewable natural resources (Álvarez & Montaña, 1997).

CONCLUSIONS

The seeds of *A. farnesiana*, *L. tridentata* and *P. laevigata* respond by increasing germination among the pooled treatments, compared to the non-pooled seed treatments. Regarding the type of soil, the germination in clay substrate was higher than that in sand. This may be because the clay soil has a great content of organic matter and also retains more moisture compared to a sandy soil. The germination speed was similar between treatments whereas between species, only *P. laevigata* responded with a higher germination when the seeds were grouped in clay soil. Therefore, the hypothesis that the separated seeds will germinate in a greater percentage independently of the substrate is rejected. Therefore, the *in situ* accumulation of seeds produces a greater germination, especially in areas where clay substrates predominate, due to its effects on the permeability of the soil.

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