

## GERMINATION OF SEVEN SPECIES OF SHRUBS IN SEMIARID CENTRAL MEXICO: EFFECT OF DROUGHT AND SEED SIZE

## GERMINACIÓN DE SIETE ESPECIES DE ARBUSTOS EN EL CENTRO SEMIÁRIDO DE MÉXICO: EFECTO DE LA SEQUÍA Y EL TAMAÑO DE LA SEMILLA

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### Abstract

**Background:** In semiarid ecosystems, many plant species are tolerant to drought. However, increased aridity as a result of climatic change could modify the capacity of germination and establishment.

**Hypothesis:** Under drought conditions, small-seeded species will tend to germinate in higher proportions than large-seeded species because the former have larger surface-to-volume ratio, allowing for more rapid water uptake.

**Study species:** *Ageratina espinosarum*, *Flourensia resinosa*, *Montanoa tomentosa* and *Gymnosperma glutinosum* (Asteraceae), *Dalea bicolor*, *Eysenhardtia polystachya* and *Mimosa pringlei* (Fabaceae).

**Study site:** Hidalgo, Mexico. September 2015.

**Methods:** We evaluated the effect of five water potential treatments on seed germination. Four dishes (replicates), each with 25 seeds, were used in each treatment. Seeds of each species were weighed and the relationship between seed germination under water stress and seed size was obtained.

**Results:** Germination decreased as water potential was reduced; almost no seeds germinated at -0.8 MPa. The least sensitive species was *Eysenhardtia polystachya*, whose germination reached 35 % at -0.6 MPa. A positive relationship was found between seed size and germination proportion under water stress.

**Conclusions:** Contrary to expectation, germination was higher in the large-seeded species in all drought treatments, suggesting that large seeds may have a greater capacity to retain water in dry environments.

**Keywords:** Polyethylene glycol, seeds, semiarid zone, water stress.

### Resumen

**Antecedentes:** En ecosistemas semiaridos muchas especies de plantas son tolerantes a la sequía. Sin embargo, el aumento de la aridez como resultado del cambio climático podría modificar la capacidad de germinación y establecimiento.

**Hipótesis:** En condiciones de sequía, las especies de semillas pequeñas tenderán a germinar en mayores proporciones que las especies de semillas grandes debido a que las primeras tienen una mayor relación superficie volumen, permitiendo la absorción de agua más rápida.

**Especies de estudio:** *Ageratina espinosarum*, *Flourensia resinosa*, *Montanoa tomentosa* and *Gymnosperma glutinosum* (Asteraceae), *Dalea bicolor*, *Eysenhardtia polystachya* y *Mimosa pringlei* (Fabaceae).

**Sitio de estudio:** Hidalgo, México. Septiembre 2015.

**Métodos:** Se evaluó el efecto de cinco tratamientos de potencial hídrico en la germinación. Se usaron cuatro cajas Petri (replicas) por tratamiento con 25 semillas por repetición. Las semillas de cada especie fueron pesadas y se obtuvo la relación entre germinación de semillas bajo estrés hídrico y tamaño de semillas.

**Resultados:** La germinación disminuyó a medida que se redujo el potencial hídrico; casi ninguna semilla germinó a -0.8 MPa. La especie menos sensible fue *Eysenhardtia polystachya*, cuya germinación alcanzó el 35 % a -0.6 MPa. Bajo estrés hídrico, se encontró una relación positiva entre el tamaño de la semilla y la proporción de germinación.

**Conclusiones:** Contrario a lo esperado, la germinación fue mayor en las especies de semillas grandes en todos los tratamientos de sequía, lo que sugiere que las semillas grandes pueden tener mayor capacidad para retener esta humedad en ambientes secos.

**Palabras clave:** Estrés hídrico, Polietilenglicol, Semillas, Zonas semi-aridas.



Arid and semi-arid ecosystems in Mexico are highly degraded and fragmented because of land use change toward crop and livestock production ([Montagnini et al. 2008](#)). Continuous perturbation in semiarid ecosystems has generated loss of fertility and soil compaction, significantly reducing the capacity of ecosystems to recover from perturbation. Moreover, the scarcity of rainfall means that recovery of the structure and functioning of arid and semiarid ecosystems; i.e., their resilience, could take decades ([Maestre 2003](#)).

Water is the most limiting resource for germination and establishment in arid ecosystems ([Noy-Meir 1973](#)), and its availability may be strongly affected by climate change ([Brown et al. 1997](#)). By 2060, average annual temperature in Mexico have been predicted to have increased by 2.3 °C and annual rainfall to have decreased by 9 % ([Sáenz-Romero et al. 2010](#)). In this context, it is a priority to select suitable species for restoration, considering not only current conditions, but also the response and anticipated adaptation to changes in rainfall patterns and the incidence of more intense droughts ([Harris et al. 2006](#)).

In arid ecosystems, many plant species can be expected to be drought-tolerant and capable of germinating under low water potentials. However, wide interspecific variation of these features have been reported ([Metz et al. 2010](#), [Merino-Martin et al. 2017](#)). Sensitivity to drought conditions depends on multiple factors and may be higher during germination and establishment ([Fischer & Turner 1978](#)). During germination, more tolerant plants have an advantage because they can establish in zones where others cannot ([Leishman & Westoby 1994](#), [Radhouane 2007](#)). Identifying drought-tolerant plants in the earliest phase of development is an important consideration in the selection of species for conservation, management and restoration efforts ([Cochrane et al. 2014](#)).

Components of the life history of plants, such as seed size, are significantly associated with plant height, growth form and dispersal mode, and can also influence recruitment patterns ([Leishman & Westoby 1994](#)). Seed size has a high impact on capacity and time of germination, establishment and survival of seedlings during early stages of the life cycle, when plants are more susceptible ([Baskin & Baskin 1998](#)). In general, average germination time tends to be greater for larger-sized seeds ([Norden et al. 2009](#)), while small seeds absorb water faster than large seeds because of their larger surface-area-to-mass ratio ([Kikuzawa & Koyoma 1999](#)).

Some studies have evaluated seed germination under drought conditions; however, the importance of seed size has received little attention in semiarid plants ([De Villalobos & Peláez 2001](#), [De la Barrera & Nobel 2003](#), [Flores & Briones 2001](#), [Van den Berg & Zeng 2006](#), [Merino-Martin et al. 2017](#)). In general, higher germination

rates occur within a range of water potentials from 0 to - 0.5 MPa. The effect of seed size has been analyzed mainly in commercial species ([Al-karaki 1998](#), [Almansouri et al. 2001](#), [Kaydan & Yagmur 2008](#), [Gholami et al. 2009](#)). Recent studies with wild species reported the unequivocal role of seed size in germination in semi-arid environments. For example, [Kidson & Westoby \(2000\)](#) argued that larger seeds are able to germinate better under drought, while [Merino-Martin et al. \(2017\)](#) reported that most light seeds had higher germination and emergence under dry conditions than heavier seeds. The authors recognize that more studies are required in order to understand whether the role of seed size in germination response under drought is general or species-specific.

We evaluated seed germination in seven shrub plants abundant in a semiarid scrub in central Mexico ([Gelviz-Gelvez & Pavón 2013](#)). These species have ecological attributes that make them suitable for potential use in ecological restoration: high frequency, considerable land cover and a high level of sociability ([Gelviz-Gelvez et al. 2014](#)). The latter attribute denotes the distribution pattern and ability of a species to associate with others rather than forming pure stands ([Wittaker 2012](#)). These characteristics can facilitate the establishment of species in advanced successional stages ([Gelviz-Gelvez et al. 2014](#)) and positive interaction with mycorrhizal fungi ([Camargo-Ricalde et al. 2002](#), [Montaño-Arias et al. 2008](#)). On the other hand, several shrub plants appear not to decrease their potential distribution under conditions of climate change when evaluated by potential distribution modeling ([Gelviz-Gelvez et al. 2014](#)). Given the potential role of these species in restoration, it is important to test whether their seeds are tolerant to water stress and whether their seed size affects germination. We hypothesize that under drought conditions, small-seeded species will germinate more quickly and in higher proportions than large-seeded species, because the higher surface-area-to-volume ratio of their seeds enables more rapid and efficient water uptake. This study may help further our understanding of the recruitment of valuable species for restoration and may help select those most suitable for germination under different drought intensities.

## Materials and methods

We selected seven shrub plants typical of semi-arid environments of central Mexico, and of ecological importance in these communities, given their high abundance and potential role in ecological restoration (coverage, density, frequency, and sociability, and the presence of mycorrhizal association), and because of their potential resilience to climate change conditions ([Gelviz-Gelvez et al. 2014](#)). The species are *Ageratina espinosarum* (A. Gray) R. M. King & H. Rob., *Flourensia resinosa*

(Brandegee) S. F. Blake., Zucc., *Montanoa tomentosa* Cerv., *Gymnosperma glutinosum* (Spreng.) Less (Asteraceae), *Dalea bicolor* Humb. & Bonpl. Ex Willd., *Eysenhardtia polystachya* (Ortega) Sarg., and *Mimosa pringlei* S. Watson (Fabaceae).

The sites where we collected the seeds of the all species, is in the semiarid shrubland of Central Mexico ( $19^{\circ} 50' - 20^{\circ} 40' N$  and  $98^{\circ} 35' - 99^{\circ} 25' W$ ), with an annual mean temperature between 15 and 19 °C and an annual precipitation of less of 450 - 700 mm. for a more detailed description of the study sites see [Gelviz-Gelvez & Pavón \(2013\)](#). The semiarid shrubland is characterized by *Acacia farnesiana*, *Celtis pallida*, *Cordia boissieri*, *Dalea bicolor*, *Eysenhardtia polystachya*, *Forestiera angustifolia*, *Gymnosperma glutinosum*, *Karwinskia humboldtiana*, *Lantana involucrata* and *Montanoa tomentosa*, among others ([Gelviz-Gelvez & Pavón 2013](#)). Our field observations indicate that all seven species produce fruits between May and December; however, phenological data by species is not available in our study or in the literature. The only exception is *E. polystachya*, which bears fruit during November-December ([Calderón-de Rzedowski & Rzedowski 2005](#)).

At each site about 500 ripe fruits were collected from at least 20 healthy individuals per species in natural populations in five localities of Hidalgo state, Mexico: Ixmiquilpan, Zimapán, Pachuca, Mextitlán and Tula, during July and September 2015. Seeds were removed from the fruits, selecting only those that presented no apparent damage, and were stored in paper bags in the dark until processed. The seeds were sterilized with 0.5 % sodium hypochlorite for 5 min., washed with deionized water and exposed to air until their surface was dry.

Mean seed size was obtained by weighing five subsamples of 100 seeds per species. Each subsample was weighed using an analytical balance (OHAUS Adventure model, USA) with  $\pm 0.0001$  g accuracy. We considered small seeds those weighing less than 500 mg. Five species had small seeds (seed size ranging from 30 to 187 mg); *A. espinosarum* ( $30 \pm 6.97$  mg), *G. glutinosum* ( $118.7 \pm 40.7$  mg), *M. tomentosa* ( $143 \pm 8.6$  mg) and *D. bicolor* ( $169.9 \pm 8.9$  mg) and three species had large seeds (seed size ranging from 500 to 948 mg); *F. resinosa* ( $548 \pm 24.28$  mg), and *E. polystachya* ( $786 \pm 69.8$  mg), and *M. pringlei* ( $948 \pm 23.53$  mg). Seed traits such as the shape or presence of dispersal structures could be important for germination. The seeds of *M. tomentosa*, *A. espinosarum* and *G. glutinosum* have elongated achenes ([Rzedowski & Calderón-de Rzedowski 1985](#)). Seeds of *Gymnosperma glutinosum* have wind dispersal syndrome ([Jurado & Estrada 2001](#)) and *Ageratina* spp. seeds have anemochory dispersal syndrome ([Cortés-Flores \*et al.\* 2013](#)). Seeds of *Eysenhardtia polystachya* ([Jurado & Estrada 2001](#)),

*Flourensia* ([Valencia-Díaz & Montaña 2003](#)), *Mimosa* spp. ([Jurado & Estrada 2001](#)), *Montanoa* spp. and *Dalea* spp. ([Cortés-Flores \*et al.\* 2013](#)) have autochorous seeds.

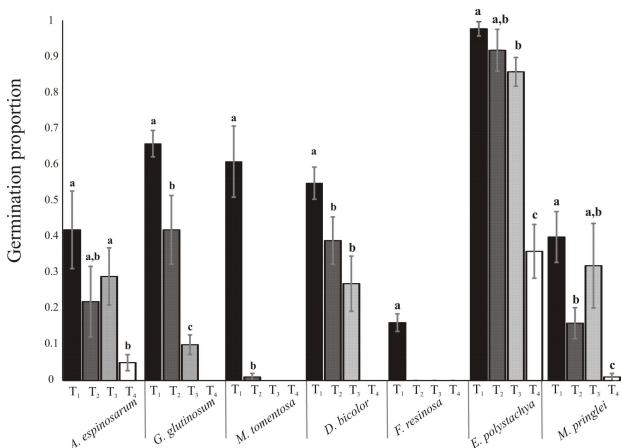
We assessed germination of all species once the seeds had been collected and dried. Germination was carried out from September to November 2015. We used a pre-germination treatment for species with low seed germination (*M. pringlei* and *D. bicolor*) by rubbing them against sandpaper to expose the embryo without damaging it, following the method of [Flores & Briones \(2001\)](#). The other species received no pre-germination treatment. Seeds of the seven selected species were placed in Petri dishes with three layers of filter paper (Whatman No. 1) as a substrate for germination. The experiment consisted of control treatments ( $T_1 = 0$  MPa) and four water potential treatments ( $T_2 = -0.2$  MPa,  $T_3 = -0.4$  MPa,  $T_4 = -0.6$  MPa and  $T_5 = -0.8$  MPa), which were obtained using polyethylene glycol (PEG 6000) with different water potentials. The concentrations used for the different water potential levels were determined based on [Villela \*et al.\* \(1991\)](#). For each treatment, the PEG-6000 was diluted in 1 L of distilled water in the appropriate proportion and stirred for 16 hours. The amount of PEG-6000 used for the treatments was 119.5 g for  $T_2$ , 178.3 g for  $T_3$ , 223.6 g for  $T_4$ , and 261.9 g for  $T_5$ . Five replicates were conducted, with 20 seeds each, for a total of 100 seeds per species per treatment. Distilled water was used as the control, since this has the maximum water pressure value (0 MPa). All seeds were placed within a plant growth chamber (VWR model 2015) at a temperature of  $25 \pm 2$  °C and 12 h light/12 h darkness. Germination was recorded daily for a period of one month, which is the recommended time for germination tests ([Baskin & Baskin 1998](#)). We considered a seed to have germinated when the emerged radicle reached  $\geq 2$  mm in length ([Iqbal \*et al.\* 2019](#)).

After the month of evaluation, we calculated the germination proportion in each treatment. Once the experiment was completed, seeds that had not germinated were opened and observed under a stereomicroscope in order to evaluate the state of the endosperm and embryo ([Ooi \*et al.\* 2004](#)).

Data for the -0.8 MPa treatment were not modelled because the majority of species did not germinate, except *E. polystachya* of which a few seeds germinated. The relationship between seed size and seed germination under drought was evaluated using a generalized linear model (GLM) with binomial error, considering water potential as a factor with four levels, and mean seed size per species as the regressor variable. To determine in what treatment, the germination was significant in relation to seed size, we used the “ggscatter” function for Pearson's correlations, considering significance at  $p = 0.05$ . All statistical analyses were conducted using R version 3.30 ([R Core Team 2016](#)).

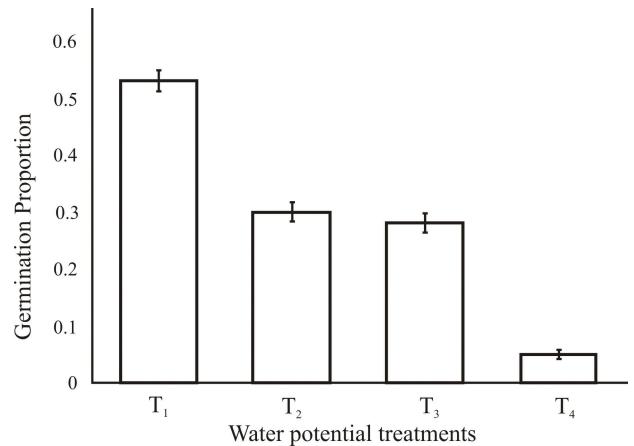
## Results

We tested viability by dissection and found that ungerminated seeds of all species were viable. Germination in the control treatment ( $T_1$ ) was highest for all seven species and decreased water potential produced a decrease in germination in all of them. This general effect was detected for each particular species and was less gradual for two taxa, *A. espinosarum* and *M. pringlei*, which presented a large variation in germination proportion between the intermediate treatments (-0.2, -0.4 MPa) (Figure 1). Germination proportion (bringing all the species together for each treatment) were different, indicating they differed in their sensitivity to drought (Figure 2). For example, *E. polystachya* was the least sensitive, having a 66 % reduction in germination at -0.6 MPa, while *G. glutinosum*, *M. tomentosa*, *D. bicolor* and *F. resinosa* had 100 % reduction at the same water potential, thus being the most sensitive species to drought.



**Figure 1.** Mean germination proportion and standard error values of the seven shrub plants under four water potential treatments:  $T_1$ ) control,  $T_2$ ) seed germination at -0.2 MPa,  $T_3$ ) seed germination at -0.4 MPa, and  $T_4$ ) seed germination at -0.6 MPa. Letters (a, b, c, d) denote significant differences.

Significant effects of water potential, seed size and their interaction were detected in the germination proportion (Table 1). For the control, seed size did not affect germination; however, under drought conditions, germination proportion increased with seed size and this effect became more pronounced with increased drought condition, as demonstrated by an increase in both the slope and significance of the relationships (Table 2, Figure 3). Overall, seed germination was driven much more strongly by water potential treatment than by seed size, with these two factors explaining 29 and 7 % of the total variance respectively (Table 2).



**Figure 2.** Mean germination proportion and standard error for all individuals that germinated by treatment.  $T_1$ ) control,  $T_2$ ) seed germination at -0.2 MPa,  $T_3$ ) seed germination at -0.4 MPa, and  $T_4$ ) seed germination at -0.6 MPa.

## Discussion

As expected, low water availability limited seed germination in semiarid plant species. However, some species were able to germinate at low water potentials: *E. polystachya*, *A. espinosarum* and *M. pringlei* had relatively high germination at -0.2 to -0.6 MPa. These species could therefore be successful in environments with low water availability, as [Briedé & Mckell \(1992\)](#) found in seven perennial arid land species subjected to moisture stress.

Our results do not support the hypothesis that small-seeded species germinate more than large-seeded species under drought conditions, as found by [Merino-Martín et al. \(2017\)](#). Our results showed under drought conditions, large-seeded species exhibited higher germination (*E. polystachya* and *M. pringlei*), in contrasts with small-seeded species.

**Table 1.** Coefficients for water potential treatments and interaction with seed size derived from the GLM binomial model. Water potential treatments:  $T_2 = -0.2$  MPa,  $T_3 = -0.4$  MPa,  $T_4 = -0.6$  MPa. The significance value was  $< 0.001$ .

	Estimate	z value	p
<b>Intercept</b>	0.1275	0.447	0.655
$T_2$	0.838	1.964	0.051
$T_3$	1.542	3.310	< 0.001
$T_4$	4.24	3.808	< 0.001
<b>Seed size * <math>T_2</math></b>	-0.011	-0.135	0.892
<b>Seed size * <math>T_3</math></b>	0.130	-1.482	0.140
<b>Seed size * <math>T_4</math></b>	0.2330	-1.434	0.153

**Table 2.** Paired comparisons of seed size effects on germination among water potential treatments for seven shrub plants. Water potential treatments:  $T_1 = 0$  MPa,  $T_2 = -0.2$  MPa,  $T_3 = -0.4$  MPa,  $T_4 = -0.6$  MPa. The significance value was  $< 0.001$ .

Contrast	Estimate	z value	p
$T_2 - T_1$	-1.338	-7.583	< 0.001
$T_3 - T_1$	-1.806	-9.647	< 0.001
$T_4 - T_1$	-4.665	-10.21	< 0.001
$T_3 - T_2$	-0.468	-2.358	0.076
$T_4 - T_2$	-3.327	-7.207	< 0.001
$T_4 - T_3$	-2.859	-6.137	< 0.001

The rationale behind the proposed hypothesis was that under natural conditions, small seeds with a high surface-area-to-volume ratio may imbibe water more quickly than large seeds with a lower ratio, particularly when periods of available soil moisture are short. However, when water potential is constantly low, higher dehydration of the seed tissues is expected in seeds with a higher surface-area-to-volume ratio, which could explain our results under conditions of constant dryness.

Other functional seed traits may have an effect on germination; *e.g.*, coat structure, shape, the presence or absence of spines, presence of endosperm, dispersal structures, tubercles or the presence or absence of mucilage (Harper *et al.* 1970, Mott 1974, Valencia-Díaz *et al.* 2015). Bu *et al.* (2016), examining 383 Alpine meadow species, found a combined effect of shape and volume of seeds on germination, where the volume of the seed had an effect on the germination of elongated seeds. In addition, the hypothesis of Merino-Martín *et al.* (2017) that small-seeded species germinate more than large-seeded species under drought could be more relevant in round seeds. We believe that more experiments considering the process of water gain and loss in seeds under heterogeneous water conditions over time are required in order to further test the role of seed size in dry environments.

The seeds of two of the seven species studied have physical latency, and mechanical scarification was used to break the dormancy of the seeds. It was considered that *E. polystachya* and *M. pringlei* had physical latency because they have an impermeable layer that prevents seed germination when scarification treatment is not applied. The layers of impervious seeds in the fabaceae can be produced by the presence of a palisade layer (Rolston 1978, Bianco & Kraus 2005), or by the presence of different substances such as cutaneous lignin, quinones, or insoluble suberine peptides that can reduce germination (Rolston 1978, Werker 1980, Valdovinos-Ponce *et al.* 1994, Sahai & Pal 1995, Baskin & Baskin 2014). Thus, seeds from all species were

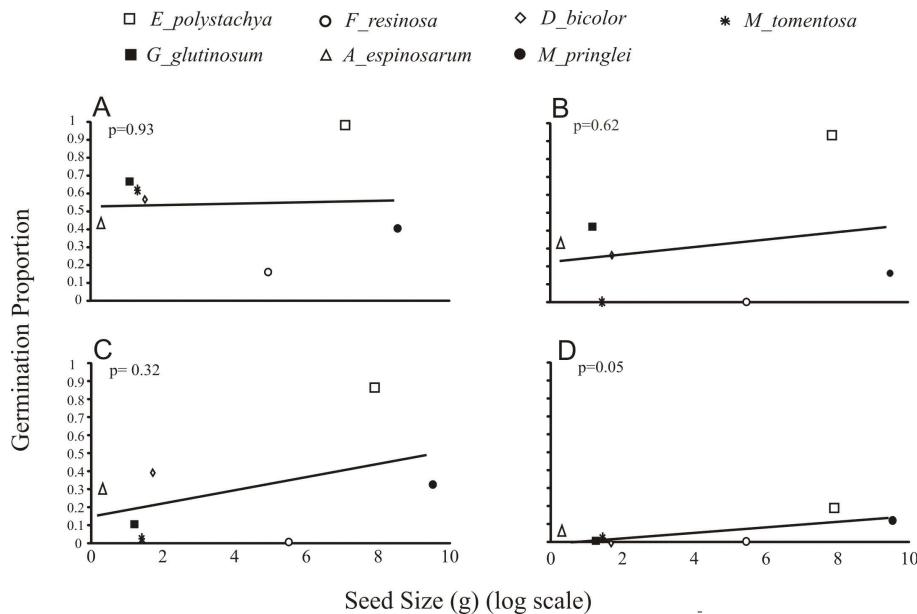
in the same condition to germinate under the water potential treatments. In addition, we found that the ungerminated seeds at the end of the experiment were viable.

Comparison of germination proportions among species can be valuable for the selection of species for restoration in the semiarid zones of central Mexico. An interesting case is that of *E. polystachya*, the species with the largest seed size, and the least affected by drought. It has a wide distribution that ranges from southeastern Arizona to southern Mexico. It is also found in different vegetation types, ranging from tropical forests to semiarid shrubland, with mean annual temperatures between 12 and 19 °C and mean annual precipitation between 300 and 1,800 mm. In addition, projections for *E. polystachya* under a higher water stress scenario than current conditions show that this species would maintain its distribution area until 2050 (Gelviz-Gelvez *et al.* 2014). Given this evidence, we consider *E. polystachya* to be an important species that is suitable for use in restoration in arid and semi-arid ecosystems of central Mexico.

On the other hand, even with its large seeds, *F. resinosa* did not respond in the same way, perhaps because some other morphological (development of the embryo) or physical (permeability of the seed coat) seed properties or physiological features that can produce dormant seed-stages and that were not measured in this study might have had a significant effect on its germination. Components of the life history of plants, including type of fruit, form of growth, color and dispersion form or agent, have been considered of great importance to find patterns of responses to environmental conditions (Cárdenas-Arévalo & Vargas-Ríos 2008). *F. resinosa* is distributed in zones with annual rainfall higher than 500 mm (Villaseñor 2016). Ford *et al.* (1983) finds that differences in the range of distribution of the species may influence germination proportion, favoring germination in species that are distributed over wide precipitation ranges. In the present study, *E. polystachya* has the widest distribution in this regard. Two species, *A. espinosarum* and *Z. augusta*, had higher germination at 0 MPa than in the other treatments. Townend *et al.* (1996) reported that some species present a reduction in germination under water stress as a strategy to reduce the risk of death during the early stages of the seedling's life cycle. Moreover, these species can develop "opportunistic" or "cautious" evolutionary strategies in sites with high environmental variability (Gutterman 2000).

In our study we found that seed size affects germination in response to drought. Different authors have discovered that seed size can be easily correlated with other functional characteristics of plants (Venable & Brown 1988, Khurana & Singh 2000, Moles & Westoby 2004, Merino-Martín *et al.* 2017) and even survival in later stages, with advantages for reproduction (Metz *et al.* 2010). For example, large seeds have the ability to store greater amounts of

## Effect of drought and seed size of shrubs in central Mexico



**Figure 3.** Correlation analysis of average seed size (g) and germination proportion of seven shrub species, under four water potential treatments: A) control, B) seed germination at -0.2 MPa, C) seed germination at -0.4 MPa, and D) seed germination at -0.6 MPa. The line indicates the level of the slope in the correlation and *p* indicates the significance level.

carbohydrate in their endosperm or cotyledons than do small seeds (Milberg & Lamont 1997). This may enable early root or photosynthetic tissue development, producing a faster-growing plant (Hewitt 1998). Khurana & Singh (2000) found that seedlings from large seeds survived extreme water stress, and have morphological and physiological mechanisms for tolerating very low soil moisture conditions over long periods that may be associated with the larger initial energy stock of the seedling.

In addition to the ecological characteristics previously evaluated, and considering the potential distribution under the most dramatic climate change scenarios (Gelviz-Gelvez *et al.* 2014), the use of species that germinate more readily under simulated drought for ecological restoration projects could reduce costs and increase establishment rates. However, more detailed studies of the germinated individuals during their early stages of development are required in order to determine whether there is a relationship between seed size and other attributes under different moisture conditions in both laboratory and field studies.

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