

Does gypsum influence seed germination?

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Abstract: Flora inhabiting gypsum outcrops in arid environments shows a high level of specialization. However, the processes involved are still unclear, specifically at the key stage of germination. Here, to assess whether gypsum could chemically influence seed germination, we tested the germination of species according to 3 functional groups: gypsophiles, gypsosvags, and calcicoles. A total of 24 taxa were selected, all occurring in gypsum and limestone substrates, under a semiarid and dry Mediterranean climate. Three levels of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) solution (low = 0.5 g/L, medium = 1 g/L, and high = 2.4 g/L) and 1 control treatment of distilled water were tested. Results depended on the particular species rather than on the functional group. We found that gypsum favored germination in some species (*Lepidium subulatum* L. and *Gypsophila struthium* L.), whereas significant negative effects appeared for only 1 species (*Rosmarinus officinalis* L.). In contrast, most of the species studied responded neutrally to gypsum solutions. Our results suggest that chemical features of gypsum could offer an advantage at the germination stage for certain species, rather than posing a constraint for seed germination.

Key words: Germination rate, gypsum solution, gypsophile, gypsosvag, calcicole

1. Introduction

Gypsum outcrops host specialized flora that appears to be more distinctive in arid and semiarid regions (Parsons, 1976; Meyer, 1986; Akpulat and Celik, 2005; Mota et al., 2011); this specialization is given even in the etymology of genus such as *Gypsophila* L. (Kormaz and Özçelik 2013). Plant species growing exclusively in gypsum soils are called gypsophiles, while others, occurring regularly in both gypsum and nongypsum soils, are called gypsosvags (Meyer, 1986). Nevertheless, many species that live in nearby habitats rarely colonize gypsum (e.g., many calcicolous plants). In this context, there is a great debate concerning the physical and chemical constraints of the flora inhabiting these areas, as well as about whether gypsophiles are refugees or specialists of gypsum substrates (Parsons, 1976; Meyer, 1986; Escudero et al., 1999, 2000; Romao and Escudero, 2005; Palacio et al., 2007).

In gypsum soils, plants encounter physical limitations such as irregular moisture distribution, high resistance to root penetration, or formation of physical soil crust (Romao and Escudero, 2005). Several studies, directly or indirectly, link gypsophily with physical factors (Meyer et al., 1992; Escudero et al., 1999). Furthermore, in

gypsum soils, the plant development could be restricted chemically from excess of sulfur and calcium (Duvigneaud and Denaeyer-De Smet, 1966; Ruíz et al., 2003), or from a nutritional impoverishment caused by the exchange of calcium for other ions retained in the soil such as nitrogen, phosphorous, and potassium (Guerrero Campo et al., 1999). Despite certain evident chemical constraints of gypsum soils for plants, the way in which gypsum chemically influences the life cycle of plants is poorly understood (Parsons, 1976; Merlo et al., 1997; Palacio et al., 2007).

Germination, a key stage in the life cycle of plants, is largely determined by temperature, water availability, and light, but also by other environmental factors such as salinity (Pujol et al., 2000). Many studies examine the influence of more soluble salts than gypsum (e.g., NaCl, CaCl_2) on germination (Tobe et al., 2003; Song et al., 2005), demonstrating that higher salinity levels usually lower the percentage of seed germination and delay the onset of the germination, or completely inhibit the process (Pujol et al., 2000). Research on these issues is prolific and, although the germination of species inhabiting gypsum environments has been the subject of some studies (Escudero et al., 1997),

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the effect of gypsum on seed germination has hardly been studied (Merlo et al., 1997).

In this paper, we tested the effect of gypsum at different concentrations on seed germination for a set of species classified in 3 functional groups according to their ability to inhabit gypsum areas: gypsophiles, gypsovags, and calcicoles. Our aim is to assess whether gypsum could influence seed germination, as well as to obtain a better understanding of the plants living on this particular geological substrate.

2. Materials and methods

2.1. Species selection and seed collection

Twenty-four taxa (Table 1) were selected and assigned to functional groups according to their edaphic preference

as follows: gypsophile, for plants restricted to gypsum soils; gypsovag, for plants that occur regularly on both gypsum and nongypsum substrates; and calcicole, for plants confined to, or most frequently found in, calcium-rich (“lime”) habitats (following Mota et al., 2011). Seeds were collected in gypsum, limestone, or both substrates in the southeastern Iberian Peninsula (37.17°N, 2.84°W), under a semiarid and dry Mediterranean climate (rainfall averaging 200 to 600 mm). Seeds were harvested from at least 50 individuals per species in natural populations from July to October 2009. Seeds were cleaned, discarding any visually malformed seeds, and were stored in darkness in paper bags under room conditions (c. 20 °C and c. 30% relative humidity) until the germination tests were started (November 2009).

Table 1. Study species assigned to functional groups by ecological preference.

Species by functional group	Abbreviations
Gypsophiles	
<i>Coris hispanica</i> Lange	Ch
<i>Gypsophila struthium</i> L. subsp. <i>struthium</i>	Gs
<i>Helianthemum squamatum</i> (L.) Dum. Cours.	Hsq
<i>Lepidium subulatum</i> L.	Ls
<i>Ononis tridentata</i> subsp. <i>crassifolia</i> (Boiss.) Nyman	Otc
<i>Ononis tridentata</i> L. subsp. <i>tridentata</i>	Ott
<i>Santolina viscosa</i> Lag.	Sv
<i>Teucrium turredanum</i> Losa & Rivas Goday	Tu
Gypsovags	
<i>Frankenia thymifolia</i> Desf.	Ft
<i>Helianthemum syriacum</i> (Jacq.) Dum. Cours.	Hsy
<i>Helianthemum violaceum</i> (Cav.) Pers.	Hv
<i>Lygeum spartum</i> L.	Lsp
<i>Pinus halepensis</i> Mill.	Ph
<i>Rosmarinus eriocalyx</i> Jord. & Fourr.	Re
<i>Rosmarinus officinalis</i> L.	Ro
<i>Stipa tenacissima</i> L.	St
Calcicoles	
<i>Cistus albidus</i> L.	Ca
<i>Cistus clusii</i> Dunal	Cc
<i>Digitalis obscura</i> L.	Do
<i>Lavandula lanata</i> Boiss.	Lln
<i>Lavandula latifolia</i> Medik.	Llt
<i>Phlomis lychnitis</i> L.	Pl
<i>Santolina chamaecyparissus</i> L.	Sc
<i>Thymus mastichina</i> (L.) L. subsp. <i>mastichina</i>	Tm

2.2. Seed-germination test

We treated seeds with 3 levels of gypsum solution (low = 0.5 g/L, medium = 1 g/L, and high = 2.4 g/L of calcium sulfate 2-hydrate [$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$] solution) and 1 control treatment with distilled water. Levels of solution were based on the maximum solubility of gypsum (2.4 g/L in water at 20 °C, Meyer, 1986). Petri dishes of 100 mm in diameter were prepared with a layer of sterile glass beads covered with a disk of filter paper. Afterwards, 25 mL of the 3 solutions or distilled water were added. The whole set-up was pasteurized before the seeds were placed in the petri dishes. Seeds were previously imbibed for 12 h and afterwards disinfected against mold with a 2% solution of commercial sodium hypochlorite for 2 min and subsequently washed with distilled water. Five replicates of 25 seeds per level of treatment and species (25 seeds \times 5 replicates \times 4 level treatment \times 24 species) were tested in a germination chamber (ASL, ± 0.1 °C) maintained at 20 °C and under 16 h light/8 h darkness. Germination, identified as visible radicle protrusion, was recorded for 60 days. The solutions were replenished when needed to avoid water restriction, replacing filter-paper disks to avoid an increase in the gypsum concentration.

Some species were pretreated to enhance seed germination: *Helianthemum* Mill. seeds were mechanically scarified by abrasion between 2 sheets of fine-grit sandpaper (Pérez-García and González-Benito, 2006), *Cistus albidus* L. seeds received a dry-heat pretreatment of 5 min at 100

°C (Escudero et al., 1997), and *Ononis tridentata* L. seeds were immersed in distilled water boiled at 100 °C and were left to cool in the water to room temperature (c. 23 °C) for 12 h (Escribá and Laguna, 2006).

2.3. Statistical analysis

We evaluated the effect of the gypsum solutions on the seed-germination rate and germination speed (as T_{50} , being the time in days needed for manifestation of half of the final germination level) by functional group, fitting generalized linear-mixed models, including gypsum treatment as a fixed factor and species as a random factor. To estimate model parameters, the Laplace approximation of likelihood was used (see Bolker et al., 2009). Generalized linear models (GLMs) were used to model effect of gypsum treatment by species. Models were fitted specifying a binomial error distribution and logit as the link function in the case of the germination rate, and Poisson error distribution and log as a link function in the case of T_{50} . All the statistical analyses were performed using the R statistical package (R Development Core Team, 2010).

3. Results

Analysis showed significant effects of gypsum on seed germination by functional group (Table 2). The 3 levels of gypsum solution had significant positive effect on the germination of gypsophile group. For gypsovag species, only the lower gypsum concentration showed a significant negative effect on seed germination. The group of calcicoles

Table 2. Generalized linear mixed model results by species group for the effect on seed germination of gypsum treatment (fixed factor). Species were included as the random factor. ¹: SG = species group, G = gypsophiles, GV = gypsovags, C = calcicoles. ²: GT = gypsum treatment. Mean values \pm SE by species group and treatment are also provided.

SG ¹	Generalized linear mixed model results					GT ²	Mean values (% \pm SE)
	Estimate	SE	z value	P (> z)			
	Intercept	0.3680	0.6775	0.543	0.5870	Control	60.80 \pm 2.62
G	Low	0.2831	0.0619	4.570	<0.0001	Low	57.00 \pm 5.39
	Medium	0.3145	0.0620	5.068	<0.0001	Medium	60.70 \pm 5.59
	High	0.1362	0.0615	2.214	0.0269	High	61.10 \pm 5.34
GV	Intercept	0.1632	0.3697	0.441	0.6589	Control	53.50 \pm 3.98
	Low	-0.1167	0.0504	-2.317	0.0205	Low	51.20 \pm 4.27
	Medium	-0.0127	0.0504	-0.252	0.8009	Medium	53.25 \pm 4.40
	High	-0.0178	0.0504	-0.354	0.7237	High	53.15 \pm 4.04
C	Intercept	0.0660	0.2251	0.293	0.7693	Control	50.46 \pm 4.01
	Low	0.1455	0.0470	3.097	0.0019	Low	54.50 \pm 3.50
	Medium	0.3447	0.0472	7.297	<0.0001	Medium	59.00 \pm 3.26
	High	0.4258	0.0474	8.983	<0.0001	High	60.80 \pm 2.62

was notable in that more seeds germinated at the highest gypsum concentration (Table 2).

Regarding the effects of gypsum solutions on germination by species, we found a significant response for some of them. However, for most species, we identified neither positive or negative significant effects, or they did not follow a pattern (Figure). Gypsum solutions favored the germination of some gypsophile species (Figure). In particular, a significantly higher number of *Lepidium subulatum* L. seeds germinated in petri dishes with gypsum (at any concentration) than without it. The seeds of *Gypsophila struthium* L. subsp. *struthium* germinated at a lower proportion under control conditions (90.4 ± 3.49). The medium level of gypsum promoted the germination rate of *Helianthemum squamatum* (L.) Dum. Cours. (44.0 ± 3.35), while the highest level of gypsum caused the lowest total germination (32.0 ± 1.79) and reduced the germination speed. Additionally, at the highest gypsum level the fewest *Coris hispanica* Lange seeds germinated (68.0 ± 3.35), while the highest germination rate for this species was reached under control conditions (74.4 ± 3.71).

For the gypsovag group, we found a positively significant effect of certain gypsum concentrations on *Pinus halepensis* Mill. and *Lygeum spartum* L. (Figure). The highest germination rate of *Pinus halepensis* seeds was reached at the medium level of gypsum (85.6 ± 5.88), significantly more seeds germinating than in the control (68.8 ± 5.28). *Lygeum spartum* germinated better at medium and high gypsum levels. On the contrary, we identified a negative effect on *Rosmarinus officinalis* L. seeds, which germinated faster (14.72 ± 0.51 days) and at a higher proportion (76.0 ± 3.10) under control conditions.

Only the seeds of genus *Lavandula* L. showed a significant effect of gypsum in the calcicolous group (Figure). In particular, *Lavandula latifolia* Medik. seeds almost failed to germinate without gypsum (3.2 ± 2.33), but they germinated at a high rate at medium (68.0 ± 7.48) and high (69.6 ± 0.98) gypsum levels.

4. Discussion

According to our results, for most species gypsum does not pose a chemical constraint at the seed germination stage. In this sense, we detected a negative response to gypsum solutions for only 1 species among the 24 studied species. In contrast, salts more soluble than gypsum have been shown in many studies to exert an inhibitory effect on germination (Pujol et al., 2000; Song et al., 2005). The effect of salts such as NaCl on seed germination has been attributed to both osmotic stress and ion toxicity (Song et al., 2005). However, the lack of a negative response to gypsum solutions in most species studied is consistent with the findings of Herrero and Porta (2000), suggesting that gypsum causes negligible osmotic stress and ion toxicity in

seed germination. Specifically, sulfates are less toxic than chlorides, probably because sulfate is a macronutrient involved in the synthesis of cell-detoxification molecules, whereas the chloride ion is a micronutrient (Léon et al., 2005). Moreover, calcium ions (Ca^{2+}) could alleviate the toxic effects of other salt components on seed germination (Tobe et al., 2003; Zehra et al., 2012). In our study, the germination analyses by functional group and gypsum treatments showed positive noteworthy effects only in the seeds of the calcicoles. This result appears to be due mainly to the strong response of *Lavandula latifolia* to the presence of Ca ions in the solution. Calcium is not only tolerated by some calcicoles but is even required by others (Clymo, 1962).

In addition, we identified a clear response to the gypsum treatments in some species. Specifically, our results suggest that some gypsophile species, such as *Lepidium subulatum* and *Gypsophila struthium* subsp. *struthium*, or *Helianthemum squamatum*, at specific concentrations could be favored during germination by the presence of gypsum. Merlo et al. (1997) also found that certain gypsum concentrations improved the germination of 2 gypsophile species. Therefore, it may be a specialization sign supporting the “specialist” model, since the soil would provide a chemical advantage for the emergence of certain gypsophiles. Consistent with this fact, other authors (Duvigneaud and Denaeyer-De Smet, 1966; Ruiz et al., 2003) pointed out other adaptations of some gypsophile species to the chemical components of gypsum soils at other life stages. Moreover, the germination of some gypsovags (i.e. *Lygeum spartum* and *Pinus halepensis*) is favored by certain gypsum concentration (especially at 1 g/L). In this sense, the role of gypsum solutions at specific concentrations would aid seed germination of some species, being useful to select appropriate conditions to promote seedling production for restoration purposes. In particular, gypsum has been found of key importance when preparing the substrate on which to perform sowings for recovery gypsum habitats (Ballesteros et al., 2012).

In contrast, we identified a negative effect of gypsum on *Rosmarinus officinalis* seeds, which germinated faster and at a higher proportion under control conditions. This gypsovag species could be favored at other stages of the cycle, developing strategies to accumulate or exclude some toxic elements characteristic of gypsum soil (Palacio et al., 2007). As an example, Romao and Escudero (2005) described a similar behavior for *Teucrium capitatum*, another gypsovag, the performance of which is hindered only in some phases by gypsum soil.

Nevertheless, the chemical features of gypsum do not seem to have a determinant effect on the germination for the overall species. The presence or absence of certain plants in gypsum outcrops may be determined by other life

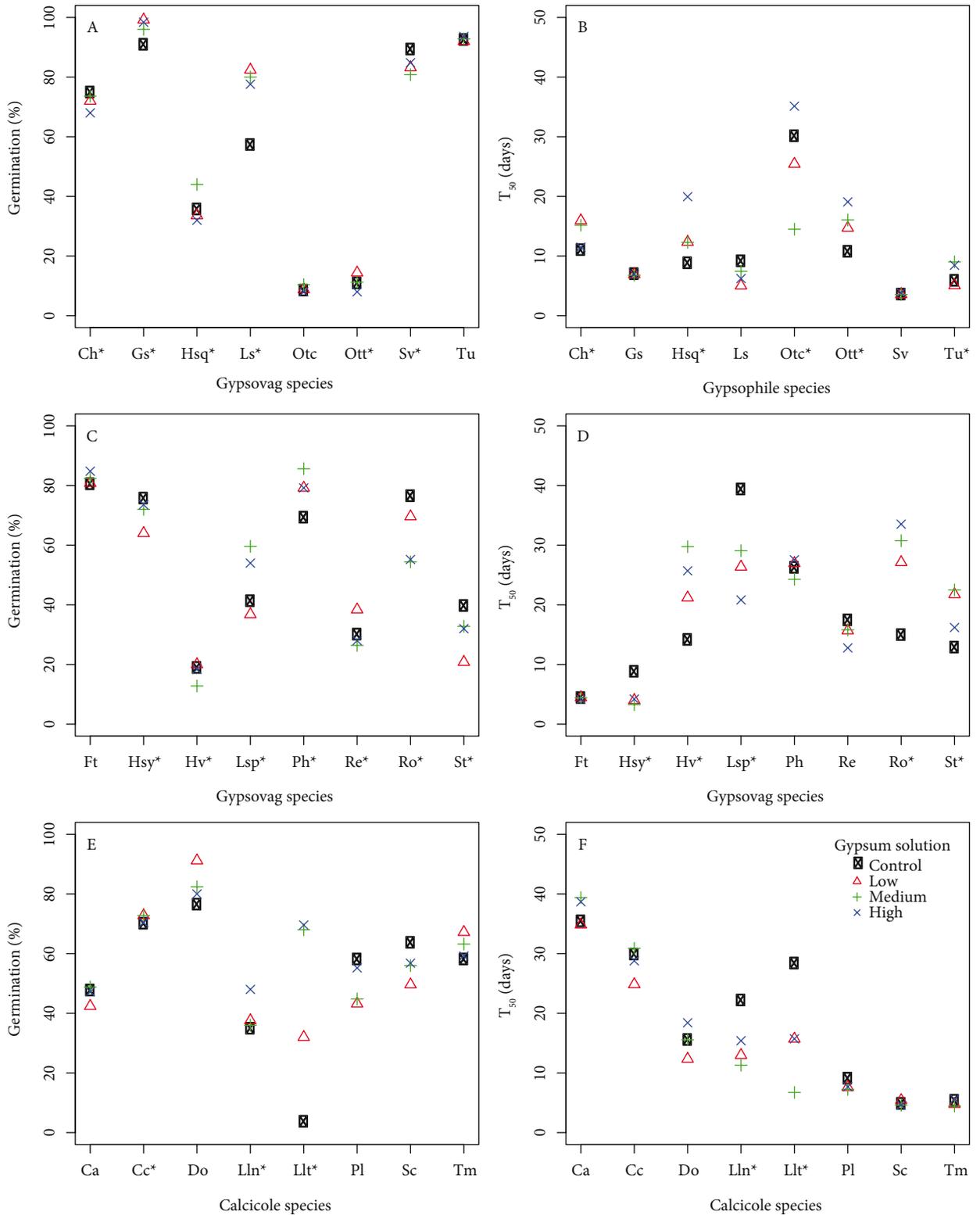


Figure. Mean values for the effect of gypsum treatment on germination (%) and T_{50} (days) by species and functional group. T_{50} : time in days needed for manifestation of half of the final germination. Species abbreviations are given in Table 1. Asterisks for the species on the x-axis indicate significant differences between treatments at $P < 0.05$ according to the GLM performed. Legend is given in F.

stages, other factors, or a combination thereof. Thus, some previous studies on the growth and survival of *Lepidium subulatum* and *Helianthemum squamatum* in gypsum soils (Escudero et al., 1999, 2000) proposed the “refuge” model for these species, linking gypsophily with some physical properties of the surface crust. Subsequently, Romao and Escudero (2005) suggested that, at least for *Helianthemum squamatum*, there is an intermediate strategy: it primarily refuges because it can penetrate gypsum crusts at the emergence stage, but it has also evolved adaptive strategies to perform better in such soils. Recently, Palacio et al. (2007), studying leaf chemical composition, suggested that regionally dominant gypsophiles (such as *Gypsophila struthium*, *Lepidium subulatum*, *Helianthemum squamatum*, and *Ononis tridentata*) might fit the ‘specialist’ model, being specifically adapted to gypsum, whereas both gypsosavgs and narrow-gypsophile endemics might fit the ‘refuge’ model, being stress-tolerant species that find refuge on gypsum soils to escape competition. This statement agrees with our results on germination: while we found positive effects of gypsum on regionally dominant gypsophiles (specifically *Gypsophila struthium*, *Lepidium subulatum*, and *Helianthemum squamatum*), we found negative or neutral effects of gypsum solution on narrow-gypsophile endemics (*Coris hispanica* and *Teucrium turredanum* Losa & Rivas Goday, respectively).

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