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Research article

Enhancing seedling production of native species to restore gypsum habitats

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ABSTRACT

Gypsum habitats are widespread globally and are important for biological conservation. Nevertheless, they are often affected by human disturbances and thus require restoration. Sowing and planting have shown positive results, but these actions are usually limited by the lack of native plant material in commercial nurseries, and very little information is available on the propagation of these species. We address this issue from the hypothesis that gypsum added to a standard nursery growing medium (peat) can improve seedling performance of gypsum species and, therefore, optimise the seedling production for outplanting purposes. We test the effect of gypsum on emergence, survival, and growth of nine native plant species, including gypsophiles (exclusive to gypsum) and gypsovags (non-exclusive to gypsum). We used four treatments according to the proportions, in weight, of gypsum:standard peat (G:S), i.e. high-g (50G:50S), medium-g (25G:75S), low-g (10G:90S), and standard-p (0G:100S).

Our results showed that the gypsum treatments especially benefited the emergence stage, gypsophiles as group, and *Ononis tridentata* as a taxon. In particular, the gypsum treatments enhanced emergence of seven species, survival of three species, and growth of two gypsophiles, while the use of the standard peat favoured only the emergence or growth of three gypsovags. Improving emergence and survival at the nursery can provide a reduction of costs associated with seed harvesting, watering, and space, while enlarging seedlings can favour the establishment of individuals after outplanting. Thus, we suggest adding gypsum to standard peat for propagating seedlings in species from gypsum habitats, thereby potentially cutting the costs of restoring such habitats. Our assessment enables us to provide particular advice by species. In general, we recommend using between 25 and 50% of gypsum to propagate gypsophiles, and between 0 and 10% for gypsovags. The results can benefit not only the production of widely distributed species commonly affected by gypsum quarrying, but also of narrow and threatened endemic species that require particularly efficient use of their seeds. In addition, our study highlights the importance of using appropriate growing media to propagate plants characteristic of special substrates for restoration purposes.

1. Introduction

Gypsum soils are widespread, with more than 100 million ha worldwide, almost exclusively in arid and semi-arid regions (Boyadgiev and Verheye, 1996). These soils host very rare and narrow endemic flora that includes many endangered species, making them priority sites for biological conservation (Anonymous, 1992; Parsons, 1976; Mota et al., 2011; Sosa and De-Nova, 2012). However, gypsum habitats are often impacted by human disturbances such as quarrying, ploughing or grazing (Al-Harthi, 2001; Mota et al., 2004; Pulido-Bosch et al., 2004; Pueyo and Alados, 2007; Ballesteros et al., 2013). Therefore, recovery plans for these environments need to be addressed, and proactive measures need to be considered (Ballesteros et al., 2012, 2014), because natural succession has proved inefficient over the short term (Mota et al., 2003, 2004; Dana and Mota, 2006).

The recovery of gypsum areas has been satisfactorily approached

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through hydroseeding (Matesanz and Valladares, 2007), sowing (Ballesteros et al., 2012) or outplanting (Sharma et al., 2001; Blignaut and Milton, 2005; Ballesteros et al., 2014). Nonetheless, one of the main problems in restoring these environments is the lack of native plant material (seeds and seedlings), even though some studies report that this is a key factor (e.g. Matesanz et al., 2006). Thus, despite the successful use of outplanting as a restoration technique for gypsum habitats (e.g. Ballesteros et al., 2014), it is difficult to find seedlings of native species for gypsum substrates (gypsum species, hereafter) in commercial or public nurseries. In fact, little information is available for producing these native species. In addition, many of the gypsum species are narrowly endemic and/or endangered species and require specific harvesting efforts and efficient use of their seeds, for which the development of effective propagation methods constitutes a priority. In this sense, testing methods are required in order to enhance the emergence and survival of seedlings. Moreover, promoting early growth of seedlings during the nursery phase is particularly relevant for better outplanting performance (Kormanik, 1986; Thompson and Schultz, 1995; Jacobs et al., 2005).

In this context, we studied seedling production in gypsum species, starting from the premise that most of these are highly specialized in gypsum substrates. In this regard, several field experiments have demonstrated that the selection of a suitable substrate, composed mainly of native gypsum, effectively contributes to the success in sowing and outplanting (Ballesteros et al., 2013, 2014). Also, other experiments evidence that the presence of gypsum in the growth medium can be a key factor for gypsum species at the initial stages (e.g. Escudero et al., 1999, 2000; Cañadas et al., 2014), but this has never been verified for seedling production. Thus, we hypothesised that the addition of gypsum to a standard growing medium could enhance seedling performance and, therefore, the production of native plants in the recovery of gypsum habitats. To test this, we designed a manipulative factorial experiment to produce seedlings of nine gypsum species in a growth chamber, adding different gypsum proportions to a nursery growing medium commonly used for plant production (peat). We monitored three key stages in plant production: emergence, survival, and early growth. Therefore, in this study, we determine whether gypsum treatments affect seedling performance, with the final aim of gaining insight into the propagation of gypsum species for habitat-restoration purposes.

2. Materials and methods

2.1. Target species and seed collection

Nine characteristic species of the EU priority habitat “Iberian gypsum vegetation, *Gypsophiletalia*” (Anonymous, 1992) were selected, including gypsophile (i.e. restricted to gypsum soils) and gypsovag plant species (i.e. occurring commonly on both gypsum and non-gypsum substrates; *sensu* Meyer, 1986). The gypsophiles were *Helianthemum squamatum* (L.) Dum. Cours. (*Cistaceae*), *Lepidium subulatum* L. (*Brassicaceae*), *Gypsophila struthium* L. subsp. *struthium* (*Caryophyllaceae*), *Ononis tridentata* L. subsp. *crassifolia* (Dufour ex Boiss.) Nyman (*Leguminosae*), and *Santolina viscosa* Lag. (*Asteraceae*). The first three gypsophiles are widely distributed in gypsum outcrops in the Iberian Peninsula and some localities in North Africa, and the last two are narrow endemic species restricted to specific gypsum outcrops in south-eastern Iberian Peninsula and considered threatened (Vulnerable; Cabezudo et al., 2005; Ballesteros et al., 2013). The four remaining species were gypsovags: *Helianthemum syriacum* (Jacq.) Dum. Cours. (*Cistaceae*), *Frankenia thymifolia Desf.* (*Frankeniaceae*), *Rosmarinus officinalis* L. *(Lamiaceae)*, *Stipa tenacissima* L. (*Poaceae*), all with a Mediterranean distribution (see Blanca et al., 2009 and Mota et al., 2011 for further details on the selected species).

Seeds were collected in gypsum outcrops in south-eastern Spain (37.17°N, 2.84°W), under a semiarid and dry Mediterranean climate (rainfall ranging from 200 to 500 mm). Seeds were harvested from at least 50 individuals per species in natural populations. Subsequently, seeds were cleaned, discarding any visually malformed seed, and stored in darkness in paper bags under ambient conditions (c. 20 °C and c. 30% relative humidity) until the experiment started.

2.2. Experimental design

We performed a manipulative experiment in a full factorial design including two factors: species (specified above) and gypsum treatments. To apply gypsum treatments, we prepared four different mixtures of standard nursery growing medium, i.e. peat (composition: organic matter = 85.4%, pH = 6–7, N = 260 mg/kg, P = 389 mg/kg, K = 2000 mg/kg, Mg = 678 mg/kg, Fe = 15 mg/kg) and powdered gypsum ($CaSO_4 \cdot 2H_2O$). According to the gypsum:standard peat (G:S) proportions in weight, we established four treatments, called: high-g (50G:50S), medium-g (25G:75S), low-g (10G:90S), and standard-p, (0G:100S, which represents the control treatment, because it is customarily used to propagate nursery plants).

Fifty replicates (pots, 6 cm × 5.6 cm × 8 cm) per treatment and species were prepared (50 pots × 4 treatments × 9 species = 1800 pots), and in each replicate ten seeds of the same species were sown. The pots were placed in a completely randomized array, in a growth chamber on three aluminium tables equipped with controlled spray-irrigation systems set to water every three days. The chamber was kept at 25 °C (ETN® thermostat, Carrier España, S.L.), under 14 h light/10 h darkness (FAEBER® lighting system, TIGER®, including 400w E40/ES OSRAM® lights, and a MicroRex D11 timer, LEXIC, LEGRAND®), reproducing favourable conditions for optimal plant development in the habitat (photoperiod and temperature from June to September).

2.3. Data collection

Pots were monitored for 21 weeks recording weekly emergence and survival. We visually checked cotyledon protrusion for emergence and marked the first seedling to emerge in each pot, or a randomly selected one if several seedlings emerged the same week (first individual, hereafter), for survival monitoring. Following the same criteria, a second seedling was marked to ensure that enough individuals were available to assess growth, in case of early death of the first individual. When each pot had two seedlings, new emerging plants were immediately clipped after recording emergence. The second marked seedling in each pot was also clipped after 4 weeks if the first individual survived, in order to avoid competition between seedlings.

After 21 weeks, the seedlings were harvested and washed with distilled water. Subsequently, we separated the shoots from roots and dried them in an oven (70 °C for 48 h). We weighed the samples in a precision scale (0.0001 g), after stabilization at room temperature, recording shoot and root biomass separately. These data were used to evaluate gypsum effects on growth.

2.4. Data analyses

The effect by species of gypsum treatments on emergence (measured as the percentage of emerged seedlings and as the time to emergence of the first individual) and growth (in terms of shoot and root biomass) was modelled by fitting generalized linear