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Distribution and germination characteristics of *Astragalus gines-lopezii*: an endangered species

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Astragalus gines-lopezii is a perennial herb, endemic to the south of the Iberian Peninsula. It has been classified as 'Endangered' by the Extremadura Regional Catalogue and placed in the equivalent category (EN D) of the National Red List because only one population in the world is known. Conservation of *A. gines-lopezii* requires a greater understanding of its population size, seed ecology and germination conditions. The latter issues are particularly important when establishing a reintroduction programme. This species was observed in nine colonies on the same mountainside, the largest of which consists of 60 plants. In this study, we compare the ability of the seeds to germinate under various conditions. Unlike most other members of the Fabaceae, the seeds of *A. gines-lopezii* do not need physical or chemical pre-treatment in order to germinate; instead they require a photoperiod for optimal germination. This study will facilitate a more in-depth understanding of this rare and poorly known species.

Key words: *Astragalus gines-lopezii*, germination treatment, seed bank, threatened species

Introduction

The genus *Astragalus* — including annuals and perennials, herbaceous plants and shrubs — is represented in the Iberian Peninsula by 41 species, and its taxa are distributed in different habitats (Podlech 1999). Nine taxa are endemic to Spain and one is endemic to the Balearic Islands. *Astragalus gines-lopezii* and *A. nitidiflorus* belong to sect. *Platyglotis* and both are endemic to Spain. The latter is endemic to Cartagena (Murcia) in the southeast (Carrión *et al.* 2007), and the former is endemic to La Parra (Badajoz) in the southwest. Both species are quite similar, and the *A. gines-lopezii* population found in Badajoz was originally described as *A.*

nitidiflorus (Vázquez *et al*. 1991), being later described as a new taxon (Talavera 1999).

The taxa present enough differences to be recognised as separate species, but at the same time they have common characteristics, belong to the same section, and are the only ones in that section that appear in the Iberian Peninsula. Furthermore, they are the most distantly located taxa of this group with the center of origin in the Near East (Talavera 1999). Species from this section exist in other Mediterranean areas: e.g. *Astragalus tuberculosus*, in northwestern Africa; *A. verucosus* in Cerdeña and *A. suberosus*. subsp. *haarbachii* in Greece (Bunge 1868).

Astragalus nitidiflorus produces up to 25 flowers per inflorescence, whereas *A. gines-lopezii* produces fewer than eight flowers per inflorescence. The fruits of both species have a coriaceous pericarp, which influences the type of seed dispersion and the manner of germination.

Astragalus gines-lopezii is catalogued as 'Endangered' by the Extremadura Regional Catalogue of Threatened Plants (DECRETO 37/2001, de 6 de marzo, por el que se regula el Catálogo Regional de Especies Amenazadas de Extremadura, Diario Oficial de Extremadura, 13 marzo 2001, número 30) and has been given the same status (EN D) in the National Red List (Moreno 2008). This taxon was not included in international or previous national red lists because of the taxonomical mistakes mentioned above. *Astragalus nitidiflorus* was catalogued as extinct (EX) by IUCN (Sánchez *et al*. 2006) and critically endangered (CR) in the National Red List (Moreno 2008).

The total number of mature *Astragalus gines-lopezii* individuals is estimated to be less than 250, all occurring in just two populations. A population is defined here as a collection of inter-breeding individuals of a particular species. A population may be composed of one or more colonies. When this study was conducted, only one population was known, but a new population was located during the spring of 2009. This population is located in the Calera Mountains at 7 km from the studied population. Both populations have the same ecological features and the new one had less than 80 individuals, all in one colony.

Despite its rarity and conservation status, no information is available on the ecology and biology of *A. gines-lopezii*. The goal of this work is thus to improve our knowledge of the ecology and reproductive biology of the population of *A. gines-lopezii*. The area was searched thoroughly in order to locate as many specimens as possible. Seeds were collected in order to characterize them, test their germination and store them in a seed bank. This work will aid our understanding of the dynamics of the population, and help to identify the main threats to its persistence.

Materials and methods

Study area

This work was carried out on a calcareous mountain in the La Parra district, in the southwest of the Iberian Peninsula (38°31´N, 6°47´W).

The site has a Mediterranean climate with the mean annual temperature of +16 °C. In winter, the mean temperature is +8 °C with –3 °C being an absolute minimum. Hot and dry summers have the mean temperature of +24.5 °C with the maximum temperature reaching +41 °C. Annual rainfall is less than 600 mm.

This region is subject to extensive agriculture. The main commercial activities are olive cultivation and livestock (sheep, pigs and cows) raising. The site where most of the colonies are situated was clear-cut three years prior. Olive trees grow at lower latitudes, and wild Mediterranean vegetation (shrubs such as *Cistus albidus*, *Teucrium fruticans*, *Phlomis purpurea* and *Quercus coccifera*, along with *Q. rotundifolia*) grows higher up where *A. gines-lopezii* was found. We found two annual species of *Astragalus*: *A. echinatus* and *A. hamosus*.

We surveyed four longitudinal, 2-km-long and 0.5-km-wide transects. Each survey was carried out by three persons walking 20 m apart. The entire potential area where the species could be encoutered had been covered. Other locations with more shrubs and animals present were excluded after we had search five randomly selected sites and found no plants. Location of each colony was recorded using GPS (Meridian platinum).

Autecology

In 2006 and 2007, the area was searched in order to identify new colonies. In each colony, *Astragalus gines-lopezii* plants were counted and classified into one of the three age groups: seedlings showing cotyledons, producer adults with old fruit or traces thereof, and non-producer adults lacking fruit and flowers. The slope, the stability of the site as well as its exposure and other characteristics were recorded for each colony.

Germination treatment

Fruits of *A. gines-lopezii* were collected from one of the colonies in May 2006. Seed availability was limited because flowering individuals and fruits per plant were few. Moreover, to avoid major damage to the populations, only a relatively small amount of fruits per plant was collected. Collection of no more than 20% of the available seed is recommended to assure natural regeneration (www.rbgkew.org.uk).

The seeds were extracted from the fruits and stored for a year in a dry and dark place. All experiments were performed in May 2007.

Seed samples were placed on two wetted filter paper discs in Petri dishes. For each treatment, there were four replicates of ten seeds per dish. Seeds were incubated for 30 days in a germination chamber (RADIBER Modelo AGP-1400-HR). Four treatments were applied:

T1 at 19 °C in darkness.
T2 at 19 °C in darkness, preceded by physical scarification of the seeds.
T3 at 19 °C in darkness, preceded by chemical scarification of the seeds by treatment with 96% sulphuric acid for 60 minutes before washing with distilled water.
T4 at 21 °C, 12 h/12 h light/dark photoperiod.

Every two days the seeds were checked to see if they had germinated. Seeds were determined to have germinated when the radical had emerged and was at least 2 mm long. For each experiment, germination percentages after 30 days were recorded. The mean germination percentage and mean germination time were then calculated.

The results were evaluated using ANOVA, a part of the SPSS 11.0 statistical software.

Results

Autecology

This study identified nine colonies of *Astragalus gines-lopezii*, eight of which were found during this study in addition to the one known previously. We use the term colonies because the plants always appear in distinct groups. Individual plants were never found in isolation between the colonies. The nine colonies were located within the area of ca. 0.5 km^2 between 500 and 700 m a.s.l. on the southern side of the mountainside.

Mature plants and seedlings were found together only in one of the colonies, while the others consisted of either mature or young plants (*see* Table 1). Five colonies had mature producer plants (bearing old fruit), whereas the other four colonies were composed of young plants or seedlings with cotyledons. These latter groups had not yet flowered.

The largest colony had 60 plants, and some of which being 19 cm in diameter. However, several plants shared lignified bases which suggests that they were old plants that had grown from a central rosette which had lost the aerial portion of the central plant.

The colonies consisting of seedlings or young plants had about 15 individuals, each colony being less than 7.5 cm in diameter. These colonies tended to be on recently ploughed soil, and one was in a trail made by runoff. The groups of mature plants occupied the most undisturbed areas.

The colonies with mature plants had only few (< 15) plants that produced fruit. The fruits (legumes) were clustered near the producer plant. Legumes were not found in the colonies of young plants.

Across the study area, this species appears where chalk comes to the surface, and seedlings were only found in those areas that had been recently ploughed by tractor. Mature plant colonies occupied the most stable, undisturbed areas, but they too were always associated with chalk near the surface. All of them were situated where lime came to the surface, on rocky soil, 5–40 cm deep, classified as brown calcareous with calcareous crust. In addition, all colonies were located on the suntrap side of the mountainside.

We also observed plants with old legumes that had been produced the previous summer. These legumes appeared close to the mother plant and presented a very hard pericarp preventing seed release. These fruits are not completely dehiscent; the valve does not split enough to release the seeds, unless water gets inside. When the fruit is humidified the seeds gain volume and they are pushed towards the small opening at the tip. Sometimes the seeds start to germinate inside the fruit.

Germination

We compared the effects of physical or chemical scarification of the seeds, and their exposure to a photoperiod on germination. The statistical analysis revealed significant differences in germination percentage among treatments ($F = 25.868$, $p < 0.001$; *see* Table 2). The highest germination rate was recorded in the photoperiod treatment (T4, $p < 0.01$), while physical or chemical scarification did not improve germination efficiency (T2, T3).

The mean germination time was shorter when seeds were scarified with sandpaper (T2; $F = 13.093$, $p < 0.01$), while the photoperiod treatments (T1 and T4) alone did not have a statistically significantly effect (*see* Figs. 1 and 2).

Discussion

Autecology

The observed spatial distribution suggests several hypotheses for the behaviour of the species. The limited dispersion of seeds was corroborated; most plants appeared grouped and not isolated. Fruit remained near their mother plants, as has been reported for other *Astragalus* species such as *A. cremnophylax* var. *cremnophylax* (Maschinski *et al*. 1997), *A. molybdenus* (Ladyman 2003), and *A. bibullatus* (Morris *et al*. 2002) that are endemic to the USA. This pattern was also reported for *A. nitidiflorus* (Carrión *et al*. 2007).

The observed distribution pattern of plants is consistent with fruits being dispersed by soil or

Table 1. Altitude, site condition, size and functional state of the nine *Astragalus gines-lopezii* colonies. The colonies are arranged following the W–E direction.

Colony	Altitude (m a.s.l.)	Site condition	Number of plants	Functional state
1	566	ploughed	16	seedlings
2	637	ploughed	12	seedlings
3	628	trail	14	mature plant
4	631	ploughed	8	seedlings
5	648	undisturbed	20	mature plant
6	661	ploughed	10	seedlings
7	650	undisturbed	10	mature plant
8	665	undisturbed	60	mature plant and seedlings
9	643	undisturbed	45	mature plant

Table 2. Percentage of germination and mean germination time of *Astragalus gines-lopezii* seeds under different germination treatments. Different letters indicate significant differences (ANOVA, $p < 0.05$).

	T1	T2	T3	T4
Germination (%)	40.00 ± 7.07^{a}	25.00 ± 16.58^{a}	2.50 ± 4.33^{b}	80.00 ± 12.25^{c}
Mean germination time (days)	10.72 ± 1.95^{a}	5.37 ± 0.37^{b}	7.00	9.48 ± 1.20^{a}

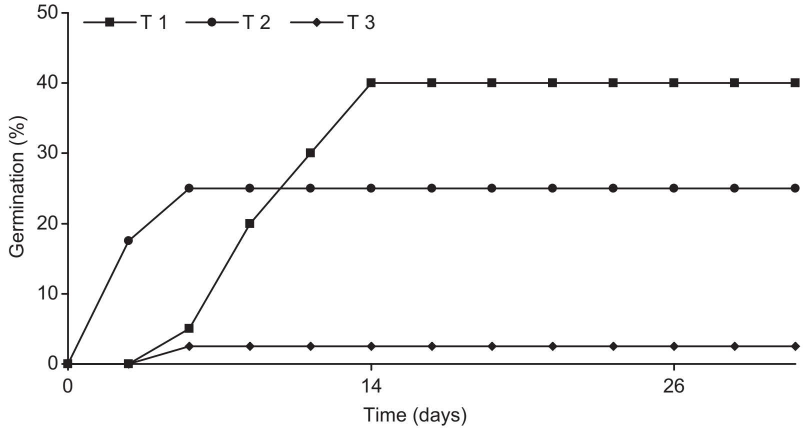

Fig. 1. Germination of *Astragalus gines-lopezii* tested in dark without pretreatment (T1) and with physical (T2) or chemical (T3) scarification.

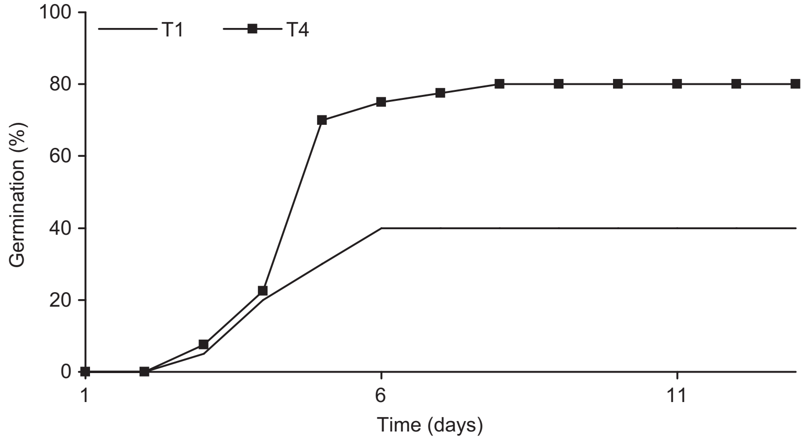

Fig. 2. Germination of *Astragalus gines-lopezii* with and without a photoperiod (T4 and T1).

hydric erosion dragging them down the slope, since some seedlings were found on the downward slope from mature plants. This clustered distribution of plants could indicate that the population was previously larger or had more mature plants. As explained above, the colony found on the trail would then have originated from seeds moved by water from mature plants situated higher up.

Because mature plants had not been previously found where there were seedlings (S. Ramos unpubl. data) and these places were ploughed by tractors, it is possible that ploughing has allowed the seeds, which lay dormant in the seed bank, to germinate. A persistent seed bank has been reported for *A. bibullatus* (Morris *et al*. 2002).

Preliminary studies suggest that *A. gines-lopezii* can tolerate at least some level of disturbance since there appears to be a persistent seed bank which appears to be one source of population stability. Morris *et al*. (2002) studied the genetic variability of the seed bank of a *A. bibullatus* population along a stratigraphic column. They found that the seeds in the deepest stratum were more variable than those above, and the seeds were more variable genetically than the individuals within the actual population. Based on this study, occasional disturbances would allow the germination of seeds produced sometime in the past, and could facilitate maintenance of genetic diversity.

These results indicate that a more detailed study on the seed bank and its genetic variability is needed.

Germination

An unexpected result was that non-scarified seeds had the highest germination rate. This is unusual for a species belonging to Fabaceae.

This family is characterized by a hard and waterproof seed coat that usually needs prior treatment in order for the seed to germinate (Baskin & Quarterman 1969, Rolston 1978, Aparicio & Guisande 1997, Baskin & Baskin 1998, Kaye 1999, Degreef *et al*. 2002, Morris *et al*. 2002, Ladyman 2003, Melgoza *et al*. 2003, Escribá & Laguna 2006, Patane & Gresta 2006, Eldredge 2007, Rincón *et al*. 2008). The related species *A. nitidiflorus* needs aggressive treatment to attain a good germination percentage (Carrión *et al*. 2007). The reasons for this behaviour might be that these seeds are included in hard legumes, so the pericarp could prevent germination, and the seed coat would not need to be hard. When the pericarp is degraded by environmental factors the seeds are released and can germinate under suitable conditions.

The positive effect of light on germination has been shown for other *Astragalus* species (Morris *et al*. 2002), but it is a less common trigger to break dormancy. Temperature and humidity have been demonstrated to be important for the germination of other *Astragalus* species (Kaye 1999). However, these factors were not tested in this study.

The presence of seedlings and young plants in recently-ploughed areas is consistent with the findings in our study. We propose that ploughing raises seeds to the soil surface so that they become exposed to light, which triggers the break in their dormancy. Ploughing would also facilitate breakdown of the pericarp and the release of seeds.

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