

Seed Germination Behaviour in *Sideritis* from Different Iberian Habitats

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Abstract

The germination behaviour of two Iberian endemic labiates, *Sideritis pungens* and *S. chamaedryfolia* selected from different habitats, ranging from very humid to semi-arid, was studied under controlled experimental conditions. A factorial experiment, combining different temperature regimes (10°, 15°, 20°, 25° or 30°C), photoperiod (total darkness and 12 h light/12 h darkness) and different pre-treatments (freezing, dry-heat, hot-water), was designed to analyze seed germination patterns and dormancy processes. The results obtained are in agreement with the ecology of the two species. Seeds are not dormant after collection and maintain high viability when preserved by standard seed bank conservation protocols. It was found out that optimal temperature for germination is 20°C for both species. Light seems to play a key role in the germination of genus *Sideritis*, particularly for *S. chamaedryfolia*, significantly inhibited by light as other psammophilous plants. Temperature and light are relevant environmental indicators, but it was found out that the germination response to these factors in the studied species is correlated with a different environmental factor, namely the availability of water resources in their habitats, which is generally a major constraint factor for seed germination in Mediterranean areas. The results indicate that germination limitations are not so much related to taxonomic position, but rather adaptations to water restrictions.

Keywords: *Labiatae*, germination tests, water availability, habitat adaptation

Introduction

Reproductive biology and particularly, seed germination is one of the phases of the plant life cycle that is most sensitive to environmental conditions. Biological cycles of endemic species are often closely adapted to their characteristic habitats. Germination behaviour, including temperature and light dependence, the rate of emergence, and dormancy occurrence may be considered a result of plant evolution to optimize survival and fitness in wild ecosystems. Most reports on seed germination have highlighted a close relationship between germination patterns of different species and environmental factors in their natural habitats (Baskin and Baskin, 1973). One decisive factor for plant survival, in periods unsuitable for the establishment and growth of new plants, such as winter or dry seasons, is the control of seed germination in relation to the most restrictive factor for this specific ecosystem (Bewley and Black, 1994). Still, light and temperature conditions are insufficient to explain germination differences registered in some cases, even in very closely related species. These environmental stimuli operate more likely as indicators of optimal conditions for an adequate development of the seedlings (Ellis *et al.*, 1985).

The cosmopolitan *Labiatae* family is especially well represented in the Mediterranean, where many of its species were traditionally used as aromatic, medicinal and

ornamental plants. One of the diversification centers of genus *Sideritis* L. is found in the Western Mediterranean and therefore many of its species are Iberian endemics.

Despite the potential commercial interest of many labiates, little is known about their optimal germination conditions and even less about the correlation of this process with their natural environments. The ecology of species belonging to this family is highly diversified, but only data on a few genera are available (Baskin and Baskin, 1998; Thanos and Doussi, 1995; Thanos *et al.*, 1995; Thanos, 2000; Pérez-García *et al.*, 2003). On the other hand, sustainable exploitation of aromatic and medicinal plants, of increasing interest nowadays, requires research related to propagation procedures and conservation of these species (Bettencourt, 1998). Also, conservation of endemics, threatened or rare plants and programmes for restoration of natural habitats need the development of optimal germination protocols.

This paper analyzes the seed germination of *Sideritis pungens* Benth., an Iberian endemic that grows in subhumid high mountains as part of supra-oromediterranean (1000-2000 m altitude) calcareous thorn-cushion vegetation and the Iberolevantine *Sideritis chamaedryfolia* Cav., characteristic of dry, thermo-mesomediterranean (500-850 m) psammophilous vegetation, which colonizes fossil dunes. The two taxa are considered rare at regional level (Laguna, 1998). *S. chamaedryfolia* is included in the red

list of Spanish vascular flora (VVAA, 2000) as vulnerable (VU).

The aim of our research is to determine the seed germination requirements and responses in the two endemics of the genus *Sideritis* mentioned above and temperature, light and heat pre-treatment are the main analyzed factors. This paper reports data on dormancy occurrence, optimal temperatures and light regime, as well as on the tolerance to heat as a type of fire adaptation. A secondary objective is to also check the effect of the preservation protocol used in our seed bank on the germination capacity of seeds, so we also analyze the response to desiccation and subsequent freezing. As a whole, our study attempts to improve the knowledge on germination strategies of Mediterranean plants.

Materials and methods

Plant material

Seeds (properly mericarps or nutlets) of *S. chamaedryfolia* were collected the 25th June of 2008 in Villena, province of Alicante, at 645 m altitude (30SXH97), with a temperate oceanic (submediterranean) bioclimate, an annual precipitation of 359 mm and a summer water deficit from May to September (Rivas-Martínez and Rivas-Sáez, 2009); *S. pungens* were collected the 10th September of 2008 from Vistabella, Penyagolosa, in the province of Castellón, at 1800 m altitude (30TYK25), with a Mediterranean pluviseasonal-oceanic bioclimate, an annual precipitation of 748 mm and a summer water deficit only during August (Rivas-Martínez and Rivas-Sáez, 2009). Voucher specimens were deposited at VAL (the Herbarium of the Botanical Garden of the University of Valencia) with the accession numbers VAL 37069 and VAL 37096, respectively. Seed samples were properly preserved in the Germplasm Bank of Wild Flora of the Valencian Community at the Botanical Garden of the University of Valencia (co-funded by the Generalitat Valenciana).

Seed sampling and storage

Mature nutlets, hereafter called seeds for simplicity, were sampled from wild plants, characterized and stored for two months in paper envelopes at room temperature and humidity (22°C, 65% RH). Seed weight was determined using an Orion Cahn C-33 microbalance (n=50). The measurements of seed dimensions (n=50) were done on a digital image using ImageJ software (ImageJ 1.42q, National Institutes of Health, USA). Prior to germination assays, the viability of seeds was checked by means of the tetrazolium test according to international guidelines (ISTA, 1999) in samples of 20 seeds per species.

Seed germination tests

We designed a factorial experiment to study the effect of different temperature and light regimes, as well as different pre-treatments on germination.

The compared conditions were: 1) control: seeds without any pre-treatment; 2) freezing: seeds were dehydrated with silica-gel, frozen, stored at -25°C for 3 months and then transferred to room temperature (22°C) in the laboratory; 3) dry-heat: seeds were spread on glass dishes and preheated at 100°C in an oven for 10 minutes; 4) hot-water: seeds were deposited in a glass tube with boiling water for 10 seconds. Pre-treated seeds were then incubated at different temperatures (10°, 15°, 20°, 25° or 30°C) under two illumination conditions: A) total darkness and B) 12 h day light photoperiod, with a mean irradiance of 25-125 $\mu\text{mol m}^{-2} \text{s}^{-1}$, provided by six cool white fluorescent lamps (General Electric F8W/33), in a cooled incubator (Sanyo Gallenkamp, Loughborough, UK).

For all treatments, four replicas of only 25 seeds were used for germination tests as very limited amount of seeds were available for collection in the natural populations of these endemic and threatened species. Seeds were placed in 9 cm glass petri dishes and germinated on one cotton disc, covered with filter paper previously moistened with 3 ml distilled water. Everyday seeds showing radicle protrusion were recorded as germinated and removed from the Petri dishes. The final germination frequency was scored after 30 days and non-germinated seeds were analyzed to identify empty or dead seeds. Surplus seeds were stored at the Germplasm Bank of the Botanical Garden, University of Valencia. Besides final germination percentages, the rate of germination was calculated according to a modified Timson's velocity index (Timson, 1965): $\sum G/t$, where G is the percentage of seeds germinated after 2 days interval and t the total time of germination (Khan and Ungar, 1984). This is an effective index to evaluate efficiency of germination because it considers not only the time to 50% germination, frequently used by other indexes, but also the final percentage of germination obtained. The highest values of the Timson's index correspond to the best conditions of germination.

Data analysis

Data were analyzed using the programme SPSS 13.5 for Windows. Germination percentages were arcsine transformed and then data were subjected to an analysis of variance.

Results and discussion

Nutlets of the two *Sideritis* species were obovate, triquetrous with prominent mid-ridge and rounded at top, coloured from pale to dark brown, darker in *S. pungens* and with speckled seeds in *S. chamaedryfolia*. The dimensions (length and width) and the mean seed weight are 2.5-2.1 x 2.0-1.6 mm and 1.88±0.57 mg for *S. chamaedryfolia* and 2.8-2.2 x 1.4-1.7 mm and 1.53±0.45 mg for *S. pungens*. Tetrazolium stain revealed high seed viability for samples of both species studied (Tab. 1).

Tab. 1. Viability percentage obtained from tetrazolium test (TZ) and mean germination percentages with their standard deviations for different light and temperature regimes and pre-treatments (ctr-control; frz-freezing; hh-humid heat; dh-dry heat) in the studied species (*Sc-S. chamaedryfolia*; *Sp-S. pungens*)

| Temperature regimes | | | 10°C | | 15°C | | 20°C | | 25°C | | 30°C | |
|---------------------|------|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | TZ | | light | darkn. | light | darkn. | light | darkn. | light | darkn. | light | darkn. |
| Sc | 100% | ctr | 9±2.0 | 12±7.3 | 29±6.8 | 65±6.8 | 60±7.3 | 99±2.0 | 11±2.0 | 44±7.3 | 5±2.0 | 41±6.8 |
| | | frz | 5±2.0 | 61±6.8 | 41±2.0 | 79±6.8 | 57±2.0 | 84±3.3 | 23±6.8 | 69±2.0 | 8±3.3 | 21±2.0 |
| | | hh | 0 | 0 | 0 | 0 | 57±2.0 | 81±6.8 | 20±3.3 | 37±2.0 | 13±2.0 | 12±7.3 |
| | | dh | 0 | 0 | 0 | 0 | 4±2.0 | 28±3.3 | 0 | 0 | 3±2.0 | 5±2.0 |
| Sp | 75% | ctr | 36±3.3 | 47±2.0 | 45±2.0 | 67±2.0 | 64±3.3 | 74±2.3 | 17±6.8 | 68±3.3 | 33±2.0 | 43±2.0 |
| | | frz | 44±7.3 | 56±7.3 | 71±2.0 | 76±3.3 | 39±2.0 | 73±2.0 | 36±7.3 | 72±3.3 | 16±3.3 | 47±2.0 |
| | | hh | 0 | 0 | 0 | 0 | 53±6.8 | 61±6.8 | 6±5.2 | 44±7.3 | 33±2.0 | 25±2.0 |
| | | dh | 0 | 0 | 0 | 0 | 19±6.8 | 61±2.0 | 0 | 0 | 20 | 24±3.3 |

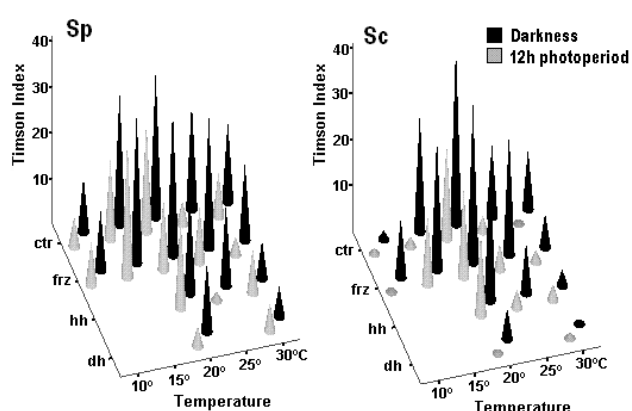


Fig. 1. Timson index mean values for the species (*Sp-S. pungens*; *Sc-S. chamaedryfolia*) and conditions studied (ctr-control; frz-freezing; hh-humid heat; dh-dry heat)

In the study of germination we found that plants from different environments developed diverse strategies. Final germination percentages, obtained under different conditions of temperature, illumination and seed pre-treatments, as well as results from viability tests are shown in Tab. 1.

The three-way ANOVA showed that germination was significantly affected by temperature, light condition, pre-treatment used and their interactions for both species (Tab. 2 and Tab. 3).

Tab. 2. Three-way ANOVA of effects of temperature, light condition, pretreatments and their interactions on seed germination of *Sideritis chamaedryfolia*

| Source | SS | d.f. | MS | F-value | P-value |
|------------------|-----------|------|-----------|---------|---------|
| Temperature (T) | 46230.600 | 4 | 11557.650 | 719.356 | 0.000 |
| Light (L) | 15523.600 | 1 | 15523.600 | 966.199 | 0.000 |
| Pretreatment (P) | 39427.600 | 3 | 13142.533 | 818.000 | 0.000 |
| T x L | 1425.400 | 4 | 356.350 | 22.179 | 0.000 |
| T x P | 20437.400 | 12 | 1703.117 | 106.003 | 0.000 |
| L x P | 7011.600 | 3 | 2337.200 | 145.469 | 0.000 |
| T x L x P | 4639.400 | 12 | 386.617 | 24.063 | 0.000 |

Darkness preference was detected in both *Sideritis* species, especially in *S. chamaedryfolia* (Tab. 1), where the final percentage of germination at 20°C in darkness was considerably higher (99%) than under light (60%).

Temperature effect on seed germination is shown in Tab. 1 and Fig. 1. Generally, 20°C represents an optimal temperature for both species, as higher and lower temperatures significantly decreased germination percentages. Significant is the fact that *S. pungens* shows high percentages in a wider range, from 15°C to 25°C. The germination rates expressed through the Timson's index are shown in Fig. 1.

Freezing and heating pre-treatments induced different responses in the studied species (Tab. 1). Freezing had no negative effects on the final germination percentages and germination rate. Seeds have showed an increase in germination capacity after storage at -25°C, except at 20°C and 30°C where some irregularities to this norm were detected. On the contrary, heat pre-treatments, both dry and humid, caused a clear inhibition of germination. These effects were accentuated under suboptimal light and temperature conditions (Tab. 1).

The first stages of the biological cycle of plants are closely dependent on water availability, particularly in the Mediterranean climate, characterized by dry summers. The plants from this region developed different germination strategies to ensure the highest survival of their seedlings. A general Mediterranean strategy is the limited op-

Tab. 3. Three-way ANOVA of effects of temperature, light condition, pretreatments and their interactions on seed germination of *Sideritis pungens*

| Source | SS | d.f. | MS | F-value | P-value |
|------------------|-----------|------|-----------|----------|---------|
| Temperature (T) | 19761.400 | 4 | 4940.350 | 353.724 | 0.000 |
| Light (L) | 9302.500 | 1 | 9302.500 | 666.050 | 0.000 |
| Pretreatment (P) | 48323.500 | 3 | 16107.833 | 1153.305 | 0.000 |
| T x L | 4205.000 | 4 | 1051.250 | 75.268 | 0.000 |
| T x P | 21245.000 | 12 | 1770.417 | 126.760 | 0.000 |
| L x P | 1981.100 | 3 | 660.367 | 47.282 | 0.000 |
| T x L x P | 5177.400 | 12 | 431.450 | 30.891 | 0.000 |

timal range of temperatures for germination, up to 20°C (Skordilis and Thanos, 1995). Baskin and Baskin (1998) gave a mean optimum germination temperature for shrubs of around 19°C. Most seeds dispersed in June and July overcome the dry season by inhibition of germination immediately after dispersion at summer temperatures. Thanos and Georghiou (1988) consider that high temperatures simply inhibit germination but do not induce any secondary dormancy. Germination is therefore possible immediately after temperature falls in autumn, which is the rainiest season in the Mediterranean region.

However seeds from less dry environments do not show a significant restriction to high temperatures. Amen (1966) reports the alpine species high germination capacity immediately after dispersal under warm temperatures. This agrees with the results of Shimono and Kudo (2005), Giménez-Benavides *et al.* (2005) and with our own results for *S. pungens*, that still reach high germination percentages at 25°C. In accordance to Thanos and Doussi (1995) this kind of response supports a spring seedling emergence and establishment for species from this kind of environment.

Another factor to be considered is that winters are mild in this region, so freezing does not usually cause secondary dormancy. In this way, emergence can extend from autumn to spring, depending on the combination of temperature, rainfall and for some species, their dormancy status.

On the other hand, heating pre-treatments induced an inhibition of germination; this is a distinctive reaction of Mediterranean species without adaptations to fire prone habitats. For Luna *et al.* (2007), recruitment of seedlings could be significantly reduced for most Mediterranean species after intense fires according to regenerative strategies and distribution range. Under this perspective, fires could be a special threat for endemics. Further investigations should be carried out with a wider range of species, to contribute to the knowledge of inhibition/tolerance/stimulation effects of fire on Mediterranean plants, much more widely studied in other areas of the world with similar climate (Keeley and Bond, 1997).

Within this basic behaviour, each species showed specific adaptations to its local environment and soil characteristics. Seed germination for the *Sideritis* species included in our study was inhibited by light, probably as an adaptation to their habitats, high mountains and sandy soils, both critically exposed to high light intensity. In many cases, light operates as a signal for the location of seeds on the soil surface, where seedlings are more sensitive to drought (Leadem, 1997). In *Sideritis pungens* the germination responses show lesser restrictions regarding the studied conditions. We associate this fact to the slighter limitations of water availability in its natural habitat. On the contrary, *Sideritis chamaedryfolia*, a typical sandy soil species, has a germination pattern strongly adapted to the edaphic characteristics. Sand substrate is particularly dry and seedlings have less survival possibilities when germination occurs at

the soil surface. The inhibition of germination in this situation is a survival factor for psammophilous species (Baskin and Baskin, 1998). Thanos *et al.* (1991) described a total or partial inhibition of seed germination on the soil surface for distinct psammophilous species. In a subsequent work, Thanos *et al.* (1994) reached the same conclusion with *Matthiola tricuspidata*.

Conclusions

The analyses of all the data concludes that both species are highly adapted to their particular habitats. The analyzed seeds do not pass through seed dormancy immediately after dispersion so they have full germination capacity when rains start in autumn. Emergence for these species is possible from autumn to spring depending on the combination of temperatures and water availability. The optimal temperature for seed germination is 20°C, but *S. pungens* shows a wider optimal range from 15°C to 25°C preferably under darkness conditions. Our study confirms that light plays a key role in germination processes in the genus *Sideritis*. Both species studied are partially inhibited by light as other taxa from similar environments.

Heat treatments have an inhibitory effect on seed germination in all the tests. The observed responses to heat of these species do not show the seed heat tolerance characteristic of fire-prone systems. Other factors, such as freezing, do not demonstrate significant effects. We consider these species orthodox, so traditional seed banking protocols are adequate to preserve seed viability in this group.

Summarizing, the seed germination process is closely related to characteristics of the habitat, mainly environmental conditions (temperature and sunlight intensity) and soil structure (drainage), which finally determines water availability, the fundamental factor for Mediterranean plants. Our results show that both species adjust to the most unfavourable factor of their characteristic habitat, which in the Mediterranean region is the summer aridity. This critical factor induces different seed germination strategies, temperature and light which are merely environmental indicators to control the germination stage.

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