NRC Research Press

ARTICLE

What can routine germination tests in seed banks tell us about the germination ecology of endemic and protected species?

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Abstract: Protocols for the conservation of threatened plants are often constrained by the absence of data on germination ecology. However, seed bank managers periodically monitor the viability of stored seed collections using germination tests. Here, we argue that data from those tests can and should be used to provide information on germination requirements of threatened species. Twelve taxa endemic to Portugal were used as a test case to determine the effect of incubation temperature and pretreatments upon germination and to identify major factors eliciting germination and releasing dormancy. We achieved maximum germination percentages >95% for nine taxa. Temperature significantly affected the final germination and mean germination time in most taxa. Maximum and faster germination at cool temperatures (15 °C or alternate 20/10 °C) was the prevailing trend. Cold stratification improved germination in one species, suggesting physiological dormancy. Scarification increased the germination percentage of one species among those expected to exhibit physical dormancy. Seed bank data provided valuable information on germination ecology, which can be used in in-situ conservation and as a baseline for further germination studies. Given the increasing threats to plant diversity, accessibility to seed bank data are paramount.

Key words: ex-situ conservation, temperature, dormancy, seed pretreatments, seed banking, threatened species.

Résumé : Les protocoles de conservation de plantes menacées sont souvent limités par l'absence de données sur l'écologie de la germination. Cependant, les gestionnaires des banques de semences suivent périodiquement la viabilité des collections de semences entreposées à l'aide de tests de germination. Les auteurs soutiennent ici que les données de ces tests peuvent et devraient être utilisées pour fournir de l'information sur les exigences des espèces menacées en ce qui concerne la germination. Douze taxons endémiques du Portugal ont été utilisés comme cas types afin de déterminer l'effet de la température d'incubation et de prétraitements sur la germination, et d'identifier les principaux facteurs qui déclenchent la germination et lèvent la dormance. Ils ont obtenu des pourcentages maximaux de germination de >95 % chez neuf taxons. La température affectait significativement la germination finale et le temps moyen de germination chez la plupart des taxons. La germination maximale et la plus rapide à des températures fraiches (15 °C ou alternées 20/10 °C) constituait la tendance dominante. La stratification froide améliorait la germination d'une espèce, suggérant l'existence d'une dormance physiologique. La scarification accroissait le pourcentage de germination d'une espèce parmi celles dont on s'attendait qu'elle présente une dormance physiologique. Les données de la banque de semences fournissent une information précieuse sur l'écologie de la germination, qui pourrait être utilisée pour la conservation *in situ*, et comme ligne de base pour d'autres études sur la germination. Compte tenu de la menace croissante qui pèse sur la diversité des plantes, l'accès aux données des banques de semences est d'une importance capitale. [Traduit par la Rédaction]

Mots-clés : conservation ex situ, température, dormance, prétraitement des semences, gestion des banques de semences, espèces menacées.

Received 8 January 2017. Accepted 28 March 2017.

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Introduction

Germination is the first fundamental stage in the life cycle of a plant. During germination, a plant is preparing for subsequent stages of establishment (further growth and development), and therefore germination is fundamental for plant adaptation (Donohue et al. 2010). The range of conditions eliciting germination of a plant determines the breadth of its niche and geographic range (Donohue et al. 2010; Luna and Moreno 2010). Among the factors that define the germination niche, temperature is of paramount importance, frequently regulating seed germination (Baskin and Baskin 1998; Probert 2000). As one of several environmental cues, temperature (including seasonal temperatures integrated by the seed over time) also plays a major role inducing or releasing seed dormancy.

Investigating germination traits of individual species is crucial for a better understanding of their ecological requirements and also to develop effective conservation and restoration protocols. Because major changes in the germination phenology and subsequent recruitment are expected under climate change scenarios (Mondoni et al. 2012; Hoyle et al. 2013; Fernández-Pascual et al. 2015), the identification of thermal thresholds for seed dormancy breaking and germination are essential to assess whether a plant is able to cope with temperature regimes that are different and, in many cases, warmer than those it is currently exposed to. Despite the large number of publications characterizing germination niches of individual species or groups of species (e.g., Baskin and Baskin 1998 and references therein; Luna and Moreno 2010), the available information is often limited for most rare and threatened species, and specific to certain floras (Crawford et al. 2007; Godefroid et al. 2010). The Secretariat of the Convention on Biological Diversity (2009) has even highlighted that ex-situ conservation is often constrained by the absence and (or) limited accessibility of data, tools, and technologies. Where information is available, a causal relationship between the rarity and the germination niche of a plant species is not evident, although often a family trend is found. On the contrary, species-specific requirements for germination are prevalent, which is frequently attributed to the specific habitats in which those species occur (e.g., high mountain habitats, specific microclimates) (Herranz et al. 2002; Giménez-Benavides et al. 2005; Lorite et al. 2007; Luna and Moreno 2010). Therefore, a thorough understanding of the germination ecology is critical to optimize conservation efforts and to contribute to a better understanding of potential species responses to climate change. Knowledge on the germination of rare and threatened species is of paramount importance for conservation practitioners, enabling them to produce plants and to increase the chance of establishment of self-sustaining populations (Merritt and Dixon 2011).

The identification of optimum germination temperatures and dormancy types requires an appropriate experimental set-up and large seed numbers (but see Baskin and Baskin 2003), which imposes further constraints to the investigation of germination traits in rare and threatened species. However, when those species are targeted for ex-situ conservation in seed banks, seed bank managers monitor the viability of stored seed collections by periodically removing samples for germination testing (Gosling 2003). Ex-situ conservation in seed banks is complementary to in-situ conservation and is effectively integrated with the protection of plants in their natural habitats and with the recovery plans of species and habitats (Offord et al. 2004; Merritt and Dixon 2011). The increasing awareness of the effects of climate change on plant distributions and the evidence for range shifts in plant populations as a consequence of climate change (e.g., Mondoni et al. 2012) has also increased the value of seed banks to study and assist in the adaptation of species to environmental change (Walck and Dixon 2009).

Seed bank routine germination tests are designed to determine the maximum potential germination and the viability of the seed accession, and their results will only provide a good indication of seed viability if species specific optimum germination conditions and methods to overcome dormancy are known (Gosling 2003; Hay and Probert 2013). Every year, seed banks perform hundreds or thousands of germination tests, and therefore effective and practical germination protocols are required. The general principle of the tests is to incubate seeds under a single, standard (preferably optimal) set of environmental conditions, to achieve the quickest, most uniform, and complete germination possible for the seed accession (Gosling 2003). When there is no previous information on the germination requirements of a species, germination conditions and pretreatments applied are usually based on information obtained from literature and on experience gained in previous tests performed on related species. Given the common limitations in seed number when handling collections of rare plants, often only one germination pretreatment is used, usually based on a "best bet" approach (Crawford et al. 2007; Martyn et al. 2009).

Although routine germination tests in seed banks do not accommodate complex experimental design to analyse germination and the complex relationship among environmental factors, dormancy, and germination traits, we argue that those tests can and should be used to provide information on germination requirements of species conserved when no other data are available. We propose that the data generated by those tests are a valuable source of information on the species' germination ecology. Using 12 taxa endemic to Portugal, our specific research aims were (*i*) to determine the effect of (*a*) incubation temperature and (*b*) pretreatments on germination and (*ii*) consequently to assess whether those routine germination tests can be employed to identify major factors eliciting germination and releasing seeds

from dormancy and to contribute to the understanding of the germination ecology of those taxa.

Material and methods

Target taxa and seed collection

We used data from routine germination tests stored in the germination database of the A.L. Belo Correia seed bank (Botanical Garden, MUHNAC, University of Lisbon, Portugal). By March 2015, this seed bank stored seeds from 61 taxa from mainland Portugal listed in the EC Habitats Directive 92/43/EEC Annexes. The majority of those were accessioned after 2009 (Clemente and Martins-Loução 2013). For this study, we selected 12 taxa (Table 1) tested for germination before seed storage. Taxa were selected according to three criteria: (*i*) at least two germination tests were performed under different conditions, (*ii*) taxa have been prioritized for national ex-situ conservation under a partnership between the A.L. Belo Correia seed bank and the national conservation agency (Instituto da Conservação da Natureza e das Florestas, ICNF), and (*iii*) taxa were endemic to Portugal. Although all selected taxa are endemic to Portugal according to the nomenclature adopted by the Habitats Directive, and used in this study, four taxa underwent relatively recent taxonomic revisions in Flora Iberica (Castroviejo et al. 1993–2013). In consequence, they have new accepted names and larger geographic ranges (*Alyssum pintodasilvae* T.R. Dudley, *Jasione crispa* (Pour.) Samp. subsp. *serpentinica* P.Silva, *Juncus valvatus* Link., and *Hyacinthoides vicentina* subsp. *transtagana* Franco & Rocha Afonso, see Table 1 for synonyms). Several taxa have restricted distribution ranges and (or) specific habitat requirements (e.g., *Convolvulus fernandesii* P.Silva & Teles, Table 1). All selected taxa grow under Mediterranean climate with a mean annual rainfall ranging from 735 to 886 mm and mean annual temperature between 12.7 and 20.8 °C (Bragança, Coimbra, Lisboa, and Setúbal weather stations; IPMA 2016).

Fully matured seeds had been collected from wild populations from 2008 to 2013, with each population and collection event representing a separate seed accession. The number of populations sampled for each taxon varied with geographic range, accuracy of information available on population localities, and resources available for seed collection. Herbarium vouchers had also been collected and are stored at the LISU Herbarium. Owing to the low seed number collected for some taxa, the number of seed accessions per taxon used in germination trials varied between 1 and 10 (Appendix A1). Seeds were maintained at room conditions (24.7 ± 1.4 °C; 52.2% ± 4.4% relative humidity) for one to two weeks before starting the germination experiments.

Germination conditions and pretreatments

Information on germination and presence of dormancy for those 12 selected taxa was surveyed to identify conditions for the initial germination tests. Published information on successful germination conditions was found for one species only (*C. fernandesii* (ICN 2007)). Therefore, type of dormancy, successful germination conditions, and pretreatments used for taxonomically related taxa sharing similar habitat and life histories were surveyed in existing seed bank databases (A.L. Belo Correia database, ENSCOBASE (2017), and Seed Information Database (Liu et al. 2008)), as well as in published literature on germination of Mediterranean species (Pérez-García et al. 1995; Escudero et al. 1997; Doussi and Thanos 2002; Copete et al. 2009). When information was available, recommended temperature regimes and pretreatments to overcome dormancy were applied. For example, physical seed dormancy is common in Convolvulaceae and Fabaceae species (González-Melero et al. 1997; Baskin and Baskin 1998); thus, a scarification pretreatment was used for taxa belonging to those families.

The effects of temperature on final germination and mean germination time (MGT) were tested according to seed availability in each seed accession. Because seed numbers differed among taxa and seed accessions within taxa, the number of temperature regimes and pretreatments tested was not consistent across taxa and seed accessions. A minimum of two and a maximum of four incubation temperature regimes were tested (15, 20, and 25 °C constant temperature and 20/10 °C alternate temperature (day/night)). Additionally, the effect of pretreatments on final germination and MGT was tested for four taxa that exhibited low germination percentages in previous trials or that were likely to be dormant. Seeds were either untreated or pretreated with one of the following dormancy-breaking treatments: cold stratification (incubation on agar at 5 °C for 56 days prior to the germination test) or scarification. Scarification was applied using one of the following methods: chipping with a scalpel, abrasion of seeds between two sheets of sandpaper, or immersion in sulphuric acid (90% H_2SO_4) for 30 min. A summary of test conditions and pretreatments applied to each seed accession is presented in Appendix A1.

A 100-seed lot was assigned to each temperature regime or temperature regime × pretreatment test and divided into four replicates with 25 seeds each, using 9 cm diameter Petri dishes with 1% agar. Petri dishes were placed in germination incubators (Fitoclima S600 and S600PL; Aralab, Lisbon, POrtugal). All germination tests were conducted in light with a 12 h photoperiod. Germination (defined as radicle emergence of approximately 1 mm) was recorded each day during the first week and afterwards every 2 or 3 days. The tests finished when no additional germination was observed for 2–4 weeks. We selected data corresponding to the minimum duration of the monitoring period of each taxon (see Appendix A1), thereby ensuring data comparability within a taxon. At the end of the germination test, a cut test determined the number of empty seeds as well as the number of firm and

Table 1. Target taxa tested for germination before storage in the seed bank.

Taxon	Family	Habitats Directive Annexes	European Red List status	Life form	Range (km²)	Dispersal (month)	Habitat
Alyssum pintodasilvae T.R. Dudley[a]	Brassicaceae	IV, V	DD	Chamaephyte	2100	July–August	Subnitrophilous dwarf shrub communities on skeletal soils derived from alkaline rocks
Convolvulus fernandesii P.Silva & Teles	Convolvulaceae	II,[e] IV	VU	Phanerophyte	100	June	Limestone walls and crevices in coastal cliffs
Hyacinthoides vicentina subsp. *transtagana* Franco & Rocha Afonso[b]	Asparagaceae	II, IV	LC	Geophyte	3600	May	Shrubland clearings and fallow land in sandy, clayey, or rocky soils with temporary waterlogging
Iberis procumbens Lange subsp. *microcarpa* Franco & P.Silva	Brassicaceae	II, IV	DD	Chamaephyte	5000	July–August	Limestone soils in coastal slopes, or near the coast
Jasione crispa (Pour.) Samp. subsp. *serpentinica* P.Silva[c]	Campanulaceae	II, IV	DD	Chamaephyte	400	July–August	Crevices in ultra-alkaline rocky outcrops, in dry areas
Juncus valvatus Link.[d]	Juncaceae	II, IV	VU	Proto-hemicryptophyte	6400	July–August	Moist meadows and runoff areas
Omphalodes kuzinskyanae Willk.	Boraginaceae	II, IV	VU	Therophyte	400	June	Shrubland clearings on sandy soils along the coast
Ononis hackelii Lange	Fabaceae	II,[e] IV	NT	Therophyte	900	May	Meadows in sandy soils, generally in pine and oak forests
Pseudarrhenatherum pallens (Link) Holub	Poaceae	II, IV	EN	Hemicryptophyte	2200	May	Soil pockets over calcareous rocks at the edges of scrublands
Santolina semidentata Hoffmanns. & Link	Asteraceae	II, IV	LC	Chamaephyte	1800	August	Shrublands on rocky outcrops and soils derived from ultra-alkaline rocks
Saxifraga cintrana Kuzinsky ex Willk.	Saxifragaceae	IV	DD	Hemicryptophyte	2100	June	Crevices in rocky outcrops and limestone walls
Silene longicilia Otth.	Caryophyllaceae	II, IV	LC	Hemicryptophyte	9900	June–August	Shrublands or rock crevices on limestone soils, as well as in marls

Note: Nomenclature according to Habitats Directive. Synonyms in *Flora Iberica* (Castroviejo et al. 1993–2013) are as follows: [a], *Alyssum serpyllifolium* Desf.; [b], *Hyacinthoides mauritanica* (Schousb.) Speta.; [c], *Jasione sessiliflora* Boiss. & Reut.; [d], *Juncus valvatus* Link var. *valvatus*.

[e]Priority species in Annex II. EN, endangered; VU, vulnerable; NT, near threatened; LC, least concern; and DD, data deficient (threat categories according to IUCN as reported in the *European Red List of Vascular Plants* (Bilz et al. 2011). Range as reported in EIONET (2015).

healthy seeds that did not germinate (Gosling 2003). Empty seeds were excluded from the analysis.

Data analysis

Percentage of germination was calculated as the number of germinated seeds at the end of the test/(initial number of seeds – number of empty seeds) × 100 (Gosling 2003). MGT was calculated as

$$\text{MGT} = \sum Dn \Big/ \sum n,$$

where n is the number of seeds that germinate on day D counted from the start of the germination test (Ellis and Roberts 1980). MGT was not calculated when germination percentage was ≤5%.

Species-specific statistic models were performed depending on the number of fixed factors tested and number of levels within fixed factors. For example, models for taxa with only one seed accession and with no pretreatment were performed including only temperature as a fixed factor. Taxa with more than one seed accession and with no pretreatment were analysed setting temperature as a fixed factor and seed accession as a random factor. When a pretreatment was applied, both temperature and pretreatment were included in the model as fixed factors (for detailed information about the number of accessions tested within taxa and the number of levels of each factor see Appendix A1).

We applied generalized linear mixed effects models (GLMMs) using Laplace approximation and binomial errors (with logit link function) to seed germination data for taxa comprising more than one seed accession (R package *lme4*; Bates et al. 2015). The GLMMs contained temperature regime as the explanatory variable and proportion of seeds germinated in each Petri dish as the response variable (coded as number of germinated seeds and number of plum seeds sown). To account for population differences in germination behaviour, seed accession was modelled as a random factor. Similarly, general linear mixed effects models (LMMs) were fitted using MGT as the response variable and a normal error distribution and temperature regime and seed accession as the fixed and random factor, respectively. When only one seed accession was available for a specific taxon, general linear models (GLMs) and linear models (LMs) were fitted to germination and MGT data, respectively, using the R package *nlme* (Pinheiro et al. 2016). Temperature or pretreatment, temperature, and the interaction term were used as fixed factors.

The effect of the fixed factors was evaluated by model selection and likelihood ratio. When overdispersion was detected, a quasibinomial error distribution was used to fit the models. Significant terms were identified using a stepwise addition to the null model, and χ^2 and F tests were used to evaluate whether selected predictors explained a significant fraction of the deviance. P values for the GLMMs were obtained using the parametric bootstrap method (R package *afex*; Singmann et al. 2015). Multiple comparisons were performed using Tukey contrasts (R package *multcomp*; Hothorn et al. 2008). All data were analysed in R version 3.2.3 (R Core Team 2015).

Results

Maximum germination percentage attained per taxa was higher than 95% for 9 of the 12 taxa (Fig. 1). Only four taxa recorded lower maximum germination percentage, *A. pintodasilvae* (72%), *C. fernandesii* (64%), *P. pallens* (69%), and *Saxifraga cintrana* Kuzinsky ex Willk. (92%). Germination was faster (MGT ≤ 5 days) in *A. pintodasilvae*, *Iberis procumbens* Lange subsp. *microcarpa* Franco & P.Silva, *Omphalodes kuzinskyanae* Willk., and *Silene longicilia* Otth and slower (MGT ≥ 10 days) in *H. vicentina* subsp. *transtagana*, *J. valvatus*, and *P. pallens* (Fig. 2).

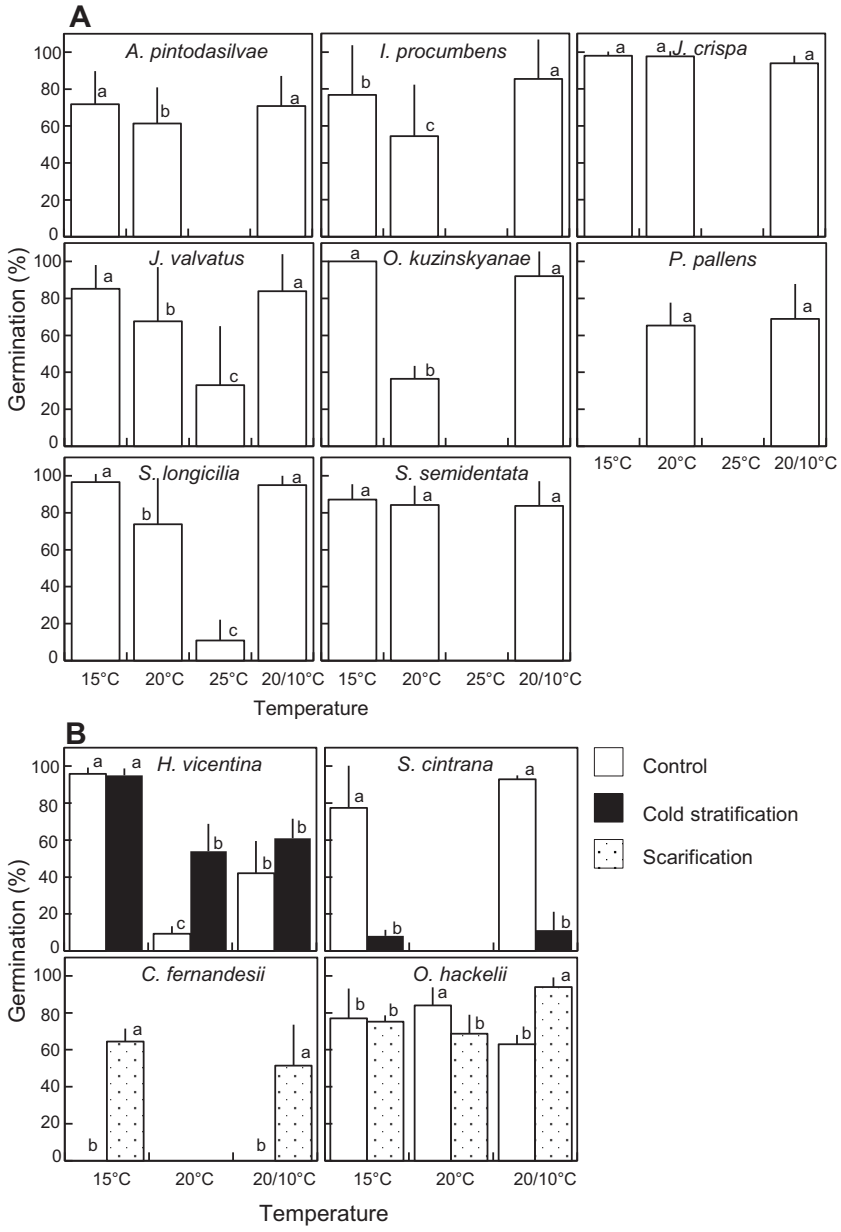
The temperature regimes tested affected the final germination of all taxa except *J. crispa* subsp. *serpentinica*, *P. pallens*, *Santolina semidentata* Hoffmanns. & Link, and *S. cintrana* (Tables 2 and 3). MGT was affected by temperature in all taxa except *A. pintodasilvae*, *O. kuzinskyanae*, and *P. pallens* (Tables 2 and 3). When the effect of temperature was significant, a generally higher and faster germination was attained at 15 °C and (or) at 20/10 °C compared with 20 °C or 25 °C (*I. procumbens* subsp. *microcarpa*, *J. valvatus*, *S. longicilia*, and *O. kuzinskyanae*) (Figs. 1 and 2). Indeed, germination was lowest and MGT highest at 25 °C in all accessions of *J. valvatus* and *S. longicilia* tested at this temperature. Only two taxa did not exhibit a decrease in seed germination at 20 °C (*J. crispa* subsp. *serpentinica* and *S. semidentata*).

The pretreatments tested had a significant effect both on final germination and on MGT but significant interactions between temperature and pretreatment were found in *H. vicentina* subsp. *transtagana* and *Ononis hackelii* Lange (Table 3). Germination of *H. vicentina* subsp. *transtagana* was highest at 15 °C, both in control and pretreated seeds, whereas germination at higher temperatures (20 °C and 20/10 °C) increased only after cold stratification (Fig. 1). This pretreatment also accelerated germination (decreased MGT) at all temperatures (Fig. 2). On the contrary, *S. cintrana* seeds pretreated by cold stratification exhibited a pronounced reduction in germination compared with control seeds, regardless of incubation temperature (Fig. 1). Seed scarification increased the germination percentage of *C. fernandesii*; germination of pretreated seeds ranged between 51% and 64% while control seeds attained only 4% (Fig. 1). In contrast, scarification with sand paper enhanced the germination of *O. hackelii* only at 20/10 °C. Seeds of this species pretreated with sulphuric acid did not germinate and were dead at the end of the test (data not shown).

Discussion

Routine seed bank germination tests proved useful for providing information on the germination ecology of the studied taxa. The use of such data when no other sources

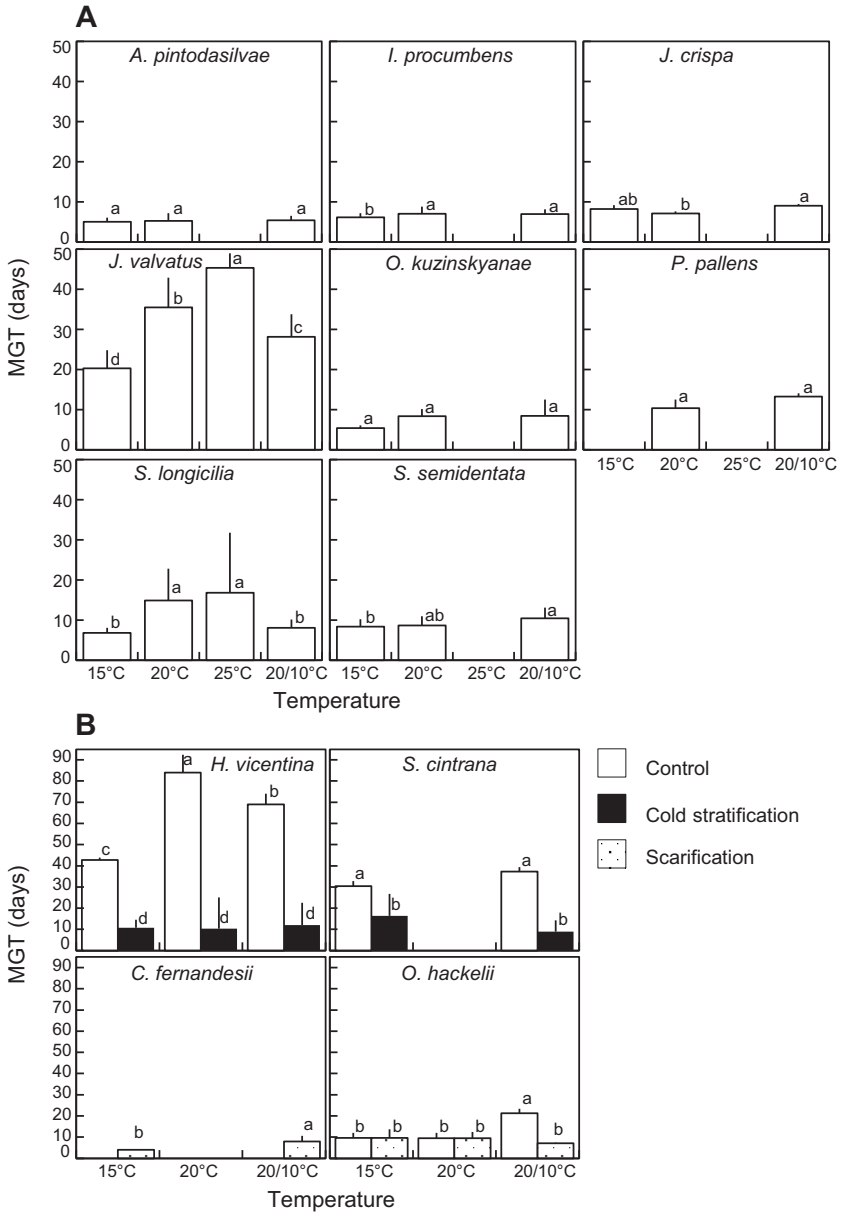
Fig. 1. Germination percentage of the studied taxa under different temperature regimes (A and B; 15, 20, and 25 °C constant and 20/10 °C day/night alternate temperatures) and pretreatments (B) (mean ± SD). Taxa means in (A) consider the variability among populations (including population as a random factor in generalized linear mixed effects models analysis). For each taxon, mean germination values for each temperature and pretreatment with different letters are significantly different ($P < 0.05$, Tukey tests).



of information exist would underpin plant production for reintroduction programmes, and the design of more comprehensive studies for a thorough understanding of germination ecology of rare and threatened species. Publishing or making the results from routine germination tests available is a prerequisite for the use of data.

Notwithstanding the relatively low seed numbers that were used (compared with studies planned to test specific hypotheses in the scientific literature (e.g., Thanos and Doussi 1995; Copete et al. 2009; Carta et al. 2013)), seed bank germination tests still evidenced major trends in optimum germination temperature and species-specific germination requirements. Our results indicate that optimal germination of most of the taxa analysed occurs at cool temperatures (15 °C or alternate 20/10 °C), as previously described for several Mediterranean species (e.g., Thanos and Doussi 1995; Escudero et al. 1997; Carta et al. 2013). However, variation in species response to temperature is evident, as for four taxa (*J. crispa* subsp. *serpentinica*, *P. pallens*, *S. semidentata*, and *S. cintrana*) the germination percentage did not vary within the range of temperature regimes tested, while for another one (*H. vicentina* subsp. *transtagana*) optimal germination occurred at lower temperatures. Requirements for dormancy breaking were

Fig. 2. Mean germination time (MGT) of the studied taxa under different temperature regimes (A and B; 15, 20, and 25 °C constant and 20/10 °C day/night alternate temperatures) and pretreatments (B) (mean ± SD). Lettering as in Fig. 1, on the basis of multiple comparison tests, after generalized linear mixed effects models analyses.



identified in three taxa, *H. vicentina* subsp. *transtagana*, *C. fernandesii*, and *O. hackelii*.

Maximum germination at cool temperatures was the prevailing trend. Suppression or reduction of germination at high temperatures could prevent germination during Mediterranean summer months, when occasional rainfall may provide a transient period of conditions suitable for germination but uncertain for seedling establishment due to drought. This strategy associates germination with autumn temperatures and the onset of rainfall, when water availability ensures seedling establishment, and is widely described for Mediterranean species (Thanos and Doussi 1995; Escudero et al. 1997; Carta et al. 2013). Indeed, even the taxa with a north-eastern distribution range (*A. pintadasilvae*, *J. crispa* subsp. *serpentinica*, and *S. semidentata*) that experience the lowest winter temperatures (IPMA 2016) did not have germination requirements similar to temperate or mountain species, which often exhibit optimal germination at high temperature or require a cold stratification period for germination (Giménez-Benavides et al. 2005). Although the timing of germination in nature is unknown for our taxa, and given the fact that the two annuals complete their life cycle in spring, it is likely that most of our taxa initiate germination in autumn or early winter, with seedling establishment and growth before the onset of summer. Most of the studied taxa colonize vegetation gaps or other open habitats. If the seed is in the appro-

Table 2. Effects of temperature regime and pretreatment on germination percentage and mean germination time (MGT).

	Germination		MGT	
Species	Model	Significance	Model	Significance
A. pintadasilvae	GLMM	$\chi^2 = 13.04$, $P = 0.0030$	LMM	$F = 0.36$, $P = 0.7020$
I. procumbens subsp. *microcarpa*	GLMM	$\chi^2 = 227.02$, $P = 0.0010$	LMM	$F = 4.17$, $P = 0.0194$
J. crispa subsp. *serpentinica*	GLM	$\chi^2 = 8.93$, $P = 0.2277$	LM	$F = 8.12$, $P = 0.0097$
J. valvatus	GLMM	$\chi^2 = 796.03$, $P = 0.0010$	LMM	$F = 184.31$, $P < 0.0001$
O. kuzinskyanae	GLM	$F = 32.80$, $P < 0.0001$	LM	$F = 1.85$, $P = 0.2116$
P. pallens	GLM	$F = 0.42$, $P = 0.5386$	LM	$F = 6.2849$, $P = 0.0461$
S. longicilia	GLMM	$\chi^2 = 1181.79$, $P = 0.0010$	LMM	$F = 19.51$, $P < 0.0001$
S. semidentata	GLMM	$\chi^2 = 0.93$, $P = 0.6100$	LMM	$F = 4.34$, $P = 0.0193$

Note: Temperature regime was modelled as an explanatory variable (fixed factor). Generalized linear mixed models (GLMM) and linear mixed models (LMM) were applied to germination percentage and MGT data, respectively, for taxa comprising more than one seed accession. Seed accession was modelled as a random factor. Generalized linear models (GLM) and linear models (LM) were applied to germination percentage and MGT data, respectively, when only one seed accession was available for the taxon. χ^2 (germination data with binomial error distribution) and F (germination data with quasibinomial error distribution and MGT data) tests and corresponding P values were used to evaluate whether selected predictors explained a significant fraction of the deviance.

Table 3. Results of the statistical analysis of the effects of temperature regime and pretreatment (scarification or cold stratification) on germination percentage and mean germination time (MGT).

Fixed factor	Germination	MGT
C. fernandesii		
Temperature	$F = 1.87$, $P = 0.1970$	$F = 9.28$, $P = 0.0226$
Scarification	$F = 94.27$, $P < 0.001$	—
Temp. × Scarif.	$F = 0.17$, $P = 0.6888$	—
H. vicentina* subsp. *transtagana		
Temperature	$\chi^2 = 44.20$, $P < 0.001$	$F = 52.00$, $P < 0.001$
Stratification	$\chi^2 = 262.62$, $P < 0.001$	$F = 1054.65$, $P < 0.001$
Temp. × Stratif.	$\chi^2 = 26.87$, $P = 0.0002$	$F = 52.67$, $P < 0.001$[a]
O. hackelii		
Temperature	$\chi^2 = 63.58$, $P = 0.8318$	$F = 8.01$, $P = 0.0032$
Scarification	$\chi^2 = 63.95$, $P = 0.1885$	$F = 18.48$, $P = 0.0004$
Temp. × Scarif.	$\chi^2 = 27.88$, $P < 0.001$	$F = 18.47$, $P < 0.001$
S. cintrana		
Temperature	$F = 3.05$, $P = 0.1060$	$F = 0.005$, $P = 0.94659$
Stratification	$F = 100.36$, $P < 0.001$	$F = 48.95$, $P < 0.001$
Temp × Stratif	$F = 0.8436$, $P = 0.3764$	$F = 5.52$, $P = 0.03679$

Note: Generalized linear models and linear models were applied to germination percentage and MGT data, respectively. Temperature regime, pretreatment, and the interaction term were included as explanatory variables (fixed factors). χ^2 (germination data with binomial error distribution) and F (germination data with quasibinomial error distribution and MGT data) tests and corresponding P values were used to evaluate whether selected predictors explained a significant fraction of the deviance.

[a]Control seeds did not germinate, test applied to the scarification treatment only.

priate microsite, germinating readily after the first rains would be advantageous for early seedling establishment and growth.

Fresh seeds of some Mediterranean species exhibit physiological dormancy immediately after collection; they require an after-ripening period to germinate and to widen the temperature range at which germination occurs (Copete et al. 2009; Mira et al. 2010), thereby preventing germination under high summer temperatures and low soil water availability. Germination of *A. pintodasilvae*, *I. procumbens* subsp. *microcarpa*, *J. valvatus*, and *S. longicilia* decreased when seeds were incubated at 20 or 25 °C, which suggests that a fraction of the seed collection might be dormant at the moment of dispersal, as shown in some of their congeners (Copete et al. 2009; Mira et al. 2010; Carta et al. 2013). Because seeds of the four taxa are dispersed from early to mid-summer, physiological dormancy would prevent germination during summer. This strategy would be particularly advantageous for *J. valvatus*, a species characteristic of temporary ponds, by preventing germination after occasional summer rainfall and coupling optimal germination temperature with waterlogged conditions (Tuckett et al. 2010; Carta et al. 2013).

Germination of *H. vicentina* subsp. *transtagana* occurred at a lower temperature (15 °C) and slower rate compared with most of the studied taxa. Cold stratification increased germination percentage and rate and widened the range of germination temperatures, indicating that seeds are dormant at dispersal, in spring. Based on the effect of cold stratification, the most probable dormancy type is physiological dormancy (sensu Baskin and Baskin 1998). However, morphological dormancy cannot be excluded because the embryo seems to have grown after stratification (personal observation). Indeed, the congeneric *Hyacinthoides non-scripta* A.S. Clemente and other related species with a similar phenology initiate embryo growth at seed dispersal but the embryo:seed ratio required for radicle emergence is attained only after exposure to low temperature (Vandelook and Van Assche 2008; Copete et al. 2011; Carta et al. 2014). Suppression of radicle emergence in a fraction of the seed lot, in combination with slow germination, may maximize the germination in waterlogged soils.

Two species were expected to exhibit physical dormancy (*C. fernandesii* and *O. hackelii*) based on the prevalence of this type of dormancy in the corresponding plant fami-

lies (González-Melero et al. 1997; Baskin and Baskin 1998). Indeed, scarification had a remarkable effect in breaking seed dormancy in *C. fernandesii*, irrespective of incubation temperature, corroborating the presence of physical dormancy in this species. Common environmental factors acting as dormancy-breaking stimuli, such as fire (e.g., Luna and Moreno 2010), are not prevalent in the coastal cliffs where this species occurs. However, exposure to high temperature and low air humidity during summer, which breaks dormancy in seeds of other Convolvulaceae (Jayasuriya et al. 2009), closely simulates the conditions that seeds of *C. fernandesii* are exposed to after dispersal in their natural environment.

Germination of *O. hackelii* was not enhanced by scarification in two out of three temperature regimes tested. These unexpected results indicate that a significant proportion of seeds do not have impermeable seed coats and germinate in the absence of dormancy breaking stimuli, as documented in other Fabaceae species (Pérez-García et al. 1995; González-Melero et al. 1997; Clemente et al. 2016) and in Cistaceae (Thanos et al. 1992).

Whilst temperature regime and pretreatments maximizing germination were identified for most taxa, the range of temperatures and pretreatments tested was limited by the available seed numbers. Therefore, expanding the range of temperatures (e.g., from 5 °C to 30 °C) (Thanos and Doussi 1995) and identifying the type of dormancy and the ecological significance of high and low temperatures during germination and dormancy breaking are required to fully analyse seed germination.

Population effects on germination characteristics were not assessed in this study because they were considered to be beyond the main objective. Nevertheless, it is worth remarking that taxa with more than three accessions (*A. pintadasilvae*, *J. valvatus*, *I. procumbens* subsp. *microcarpa*, *S. semidentata*, and *S. longicilia*) often exhibited high standard deviations in germination percentage and (or) MGT, indicating variation in population responses to temperature. Local environmental conditions affect the germination characteristics of species across a wide range of plant communities (e.g., Baskin and Baskin 1998 and references therein; Giménez-Benavides et al. 2005), and this fact highlights the importance of testing samples from individual populations for viability during seed bank monitoring, rather than assuming an identical germination behaviour among different samples of the same species. Conclusions based on data from only one population should therefore be drawn only very carefully.

We hope this study encourages seed bank managers to make their germination data widely accessible and the scientific community to use such data as baseline information to design comprehensive studies on seed germination. Some constraints inherent to the germination procedures applied in seed banks (such as limited statistical power due to low sampling sizes or the absence of replicates) may preclude the publication of part of the data in peer-reviewed journals. Nevertheless, such data can still be made available e.g., in on-line reports or databases. Databases such as RBGK's Seed Information Database (Liu et al. 2008) or ENSCOBASE (2017) are currently the largest on-line sources of information on germination requirements. ENSCOBASE currently offers data on ex-situ conservation of 11 515 wild taxa stored in 34 native seed banks across Europe, including individual 21 181 germination tests (ENSCOBASE 2017). Standardized templates and private URLs make it straightforward for seed bank managers to upload their germination data.

Taxonomic revisions and the use of different names by different seed bank managers for the same species are major constraints to data uniformization for accessibility and cause difficulty for users searching for information on a specific taxon. Many taxa have alternative names or are placed, depending on the taxonomic concept, into a different taxon, as seen for a few of our target taxa (Table 1). To allow cross-referencing between different names and taxonomic concepts, seed bank managers should try to maintain a record of all names of an accession and a list of synonyms. Selecting a prevailing authority for all accepted names and synonyms (e.g., Euro+Med PlantBase (2017); The Plant List (2017)) would also produce a taxonomically consistent list of taxa. Users should be aware of this issue and remain cautious when searching seed bank databases for information on a specific taxon.

Conclusions

Routine seed bank germination tests provided valuable baseline information on the germination ecology of the studied taxa, which is a basis for more comprehensive scientific studies. Our results are also valuable to develop conservation protocols for rare and threatened species. In general, germination percentages were high and pretreatments to break seed dormancy are easy to apply under controlled conditions, which may compensate the scant seed material available for plant production. Therefore, major efforts should be focused on those taxa that, in addition to low germination percentages, have a critical conservation status. In our set of 12 studied taxa, *C. fernandesii*, *J. valvatus*, *O. kuzinskyanae*, *P. pallens*, and *S. cintrana* had already been identified by the national conservation programme as requiring population reinforcements (PSRN2000 2008). All of those taxa reached high germination percentage except *P. pallens*, which requires additional studies to identify the optimum germination temperature.

Overall, the high germination percentages obtained indicate that the main threats to the in-situ conservation of these taxa are likely to be related to the habitat where seedlings establish or to dispersal or seed viability constraints. Studies addressing seed dispersal mechanisms,

the timing of seedling emergence and establishment in nature, and the ability to form a persistent seed bank (Copete et al. 2009) would therefore further enhance conservation and management efforts.

Given the increasing threats to plant diversity (RGB Kew 2016), it is urgently necessary to make use of seed bank germination data. We hope our study encourages other seed banks to publish or promote accessibility to their data and fosters the communication and cooperation among seed bank managers, conservation practitioners, and researchers to maximize the chances of germination of rare and threatened species and their in-situ conservation.

Acknowledgements

We acknowledge the collaboration of the Instituto da Conservação da Natureza e das Florestas and thank Domitila Brocas for laboratory assistance. We also thank three anonymous reviewers for their constructive comments on the manuscript. This work was supported by the Millennium Seed Bank Partnership, Royal Botanic Gardens, Kew; the Fundo EDP para a Biodiversidade 2009 (Fundação EDP); and the Fundação para a Ciência e a Tecnologia (BGCT/33418/2008, A.S. Clemente).

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Appendix A

Appendix Table A1 appears on the following page.

Table A1. Seed accessions tested in each taxon and conditions of the germination tests.

Taxon	Seed accession number[a]	Collecting date	Provenance	Temperature (°C)[b]	Pretreatment	Test duration (days)
A. pintadasilvae	BG.MNHN.UL 009565	16 August 2013	Bragança	15, 20, 20/10	—	45
A. pintadasilvae	BG.MNHN.UL 009572	15 August 2013	Vinhais	15, 20, 20/10	—	45
A. pintadasilvae	BG.MNHN.UL 009580	14 August 2013	Bragança	15, 20, 20/10	—	45
A. pintadasilvae	BG.MNHN.UL 009619	11 July 2013	Macedo de Cavaleiros	15, 20, 20/10	—	45
C. fernandesii	BG.MNHN.UL 009827	10 June 2011	Sesimbra	15, 20/10	Scarification[c], control	55
H. vicentina subsp. *transtagana*	BG.MNHN.UL 009649	25 May 2013	Palmela	15, 20, 20/10	Cold stratification[d], control	100
I. procumbens subsp. *microcarpa*	BG.MNHN.UL 009711	05 August 2012	Rio Maior	15, 20, 20/10	—	50
I. procumbens subsp. *microcarpa*	BG.MNHN.UL 009797	17 August 2011	Sintra	15, 20, 20/10	—	50
I. procumbens subsp. *microcarpa*	BG.MNHN.UL 009798	17 August 2011	Sintra	15, 20, 20/10	—	50
I. procumbens subsp. *microcarpa*	BG.MNHN.UL 009802	19 August 2011	Vila Franca de Xira	15, 20, 20/10	—	50
I. procumbens subsp. *microcarpa*	BG.MNHN.UL 009804	19 August 2011	Sesimbra	15, 20, 20/10	—	50
I. procumbens subsp. *microcarpa*	BG.MNHN.UL 009806	11 August 2011	Porto de Mós	15, 20/10	—	50
I. procumbens subsp. *microcarpa*	BG.MNHN.UL 009978	11 July 2008	Setúbal	15, 20, 20/10	—	50
J. crispa subsp. *serpentinica*	BG.MNHN.UL 009608	14 July 2013	Vinhais	15, 20, 20/10	—	50
J. valvatus	BG.MNHN.UL 009799	17 August 2011	Sintra	15, 20, 25, 20/10	—	55
J. valvatus	BG.MNHN.UL 009801	19- August 2011	Vila Franca de Xira	15, 20, 25, 20/10	—	55
J. valvatus	BG.MNHN.UL 009808	10 August 2011	Azambuja	15, 20, 25, 20/10	—	55
J. valvatus	BG.MNHN.UL 009810	10 August 2011	Alenquer	15, 20, 25, 20/10	—	55
J. valvatus	BG.MNHN.UL 009821	05 August 2011	Loures	15, 20, 25, 20/10	—	55
J. valvatus	BG.MNHN.UL 009823	05 August 2011	Sintra	15, 20, 25, 20/10	—	55
J. valvatus	BG.MNHN.UL 009824	04 August 2011	Sintra	15, 20, 25, 20/10	—	55
J. valvatus	BG.MNHN.UL 009911	19 July 2010	Loures	15, 20, 25, 20/10	—	55
O. hackelii	BG.MNHN.UL 009847	21 May 2011	Santiago do Cacém	15, 20, 20/10	Scarification[e], control	60
O. kuzinskyanae	BG.MNHN.UL 009941	15 June 2010	Cascais	15, 20, 20/10	—	30
P. pallens	BG.MNHN.UL 009850	05 May 2011	Alenquer	20, 20/10	—	40
S. cintrana	BG.MNHN.UL 009764	10 June 2012	Porto de Mós	15, 20, 20/10	Cold stratification[d], control	50
S. longicilia	BG.MNHN.UL 009707	07 August 2012	Arruda dos Vinhos	15, 20, 20/10	—	60
S. longicilia	BG.MNHN.UL 009714	19 July 2012	Condeixa-a-Nova	15, 20/10	—	60
S. longicilia	BG.MNHN.UL 009723	18 July 2012	Ansião	15, 20/10	—	60
S. longicilia	BG.MNHN.UL 009734	16 July 2012	Porto de Mós	15, 20/10	—	60
S. longicilia	BG.MNHN.UL 009769	02 June 2012	Loures	15, 20/10	—	60
S. longicilia	BG.MNHN.UL 009835	28 June 2011	Sintra	15, 20, 25, 20/10	—	60
S. longicilia	BG.MNHN.UL 009841	09 June 2011	Mafra	15, 20, 25, 20/10	—	60
S. longicilia	BG.MNHN.UL 009842	09 June 2011	Cadaval	15, 20, 25, 20/10	—	60
S. longicilia	BG.MNHN.UL 009843	01 June 2011	Sintra	15, 20	—	60
S. longicilia	BG.MNHN.UL 009902	14 July 2010	Figueira da Foz	15, 20, 25, 20/10	—	60
S. semidentata	BG.MNHN.UL 009567	16 August 2013	Vinhais	15, 20, 20/10	—	60
S. semidentata	BG.MNHN.UL 009571	15 August 2013	Vinhais	15, 20, 20/10	—	60
S. semidentata	BG.MNHN.UL 009578	14 August 2013	Bragança	15, 20, 20/10	—	60

Note: The provenance of each accession is indicated by the Portuguese *Concelho* geographical unit. The experimental design used for each taxon is indicated by the number of accessions tested and the levels tested within each factor (temperature and pretreatment). Four replicates of 25 seeds were used in each test (temperature level or temperature level × pretreatment level).

[a]Accession number at A.L. Belo Correia Seed Bank.

[b]Temperature regimes tested were either constant or alternate day/night temperatures under a photoperiod of 12 h light/12 h dark.

[c]Chipping with scalpel.

[d]Incubation on agar at 5 °C for 56 days.

[e]Two scarification pretreatments: abrasion of seeds between two sheets of sandpaper and immersion in 90% sulphuric acid for 30 min.