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The role of cold storage and seed source in the germination of three Mediterranean shrub species with contrasting dormancy types

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Abstract

• **Context** The use of native species in ecological restoration is highly recommended but, in practice, it is often impaired by knowledge gaps in the germination ecology of suitable species.

• **Aims** This study aimed to assess the role of storage conditions and seed source on the germination of three Mediterranean shrub species with contrasting types of dormancy.

• **Methods** Ripe fruits were harvested at two or three distant locations in mainland Portugal. Seeds were subjected to three treatments consisting in different storage conditions: cold storage at low and high moisture conditions, plus a control. Five replicates of up to 30 seeds were placed under constant

temperature conditions and germination was monitored weekly during 14 weeks.

• **Results** The effect of cold storage at high moisture on germination differed between the three species and seed source played a significant role in the germination of all three species. In the case of the species with dormancy, the observed differences in germination could reflect changes in the species' dormancy degree or sensitivity to dormancy breaking factors across their geographical range. In the case of *Pistacia lentiscus* (no dormancy), the results suggested a possible adaptation of the northern seed source to high moisture conditions.

• **Conclusions** The observed differences between species agreed well with their dormancy types, and the seed source-related differences could be adaptive features, as they seemed related with local climate conditions.

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Contribution of the co-authors Ana Vasques: Experimental design, species selection, data collection, data analysis and writing the paper.

V. Ramón Vallejo: Experimental design, species selection, intermediate reviews, logistical support, supervision.

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1 Introduction

Mediterranean plant communities are increasingly threatened by inadequate management (Díaz-Delgado et al. 2002; Pausas et al. 2008; Scarascia-Mugnozza et al. 2000). Climate change scenarios are expected to aggravate degradation processes through an increase in the frequency and severity of summer droughts and forest fires (Solomon 2007; Vallejo et al. 2012). Restoration actions involving seeding or planting are increasingly required to reverse land degradation processes (Vallejo et al. 2005).

In restoration actions, ex situ conservation protocols for native species assume a particular importance (Merritt and Dixon 2011). While the use of native species is highly

recommended, it often presents major challenges with respect to the availability of seeds as well as the definition of adequate seed harvesting, storage and seeding protocols (Tischew et al. 2011; Oliveira et al. 2011). The successful use of seeds in restoration actions critically depends on the one hand on appropriate storage methods to maintain seed viability, but on the other hand on the knowledge of the conditions that will break seed dormancy (Merritt and Dixon 2011). According to Oliveira et al. (2011), the main drawbacks for predicting germination success in many native species are the knowledge gaps regarding the presence of different types of dormancy and the correspondent dormancy breaking mechanisms.

Seed dormancy is defined as the inability of seeds to germinate under conditions that otherwise would favour their germination (Baskin and Baskin 2004). The main types of seed dormancy are physiological and physical dormancy (Baskin and Baskin 2004; Taiz and Zeiger 2010). Physiological dormancy is associated with the hormone balance of the embryo and requires specific external stimuli to be terminated, while physical dormancy is associated to the seed coat, which acts as a physical barrier, inhibiting the entrance of water into the seed (Murdoch and Ellis 2000; Finch-Savage and Leubner-Metzger 2006).

The dormancy type of a species determines its overall germination response, i.e. the type of initiation stimuli that are necessary to break dormancy and the range of temperature and water potential conditions that allows germination (Murdoch and Ellis 2000). Dormancy is not a fixed but a dynamic state that can be present in different degrees (Finch-Savage and Leubner-Metzger 2006; Long et al. 2014). The degree of dormancy (in the case of physiological dormancy) or the sensitivity to dormancy breaking factors (in the case of physical dormancy) can vary in response to environmental stimuli during seed development as well as following dispersal (Long et al. 2014). A greater sensitivity to dormancy breaking factors implies that a broader range of conditions, including in terms of temperature and water potential, can induce germination (Fenner and Thompson 2005; Long et al. 2014).

Differences in dormancy have been invoked to explain differences in germination, between seeds of the same fruit and also from different fruits, mother plants and plant populations (Murdoch and Ellis 2000). In general, warmer conditions during seed development lead to a lower degree of dormancy (Long et al. 2014). In this sense, seeds from populations with distinct climate conditions have been found to differ in germination (Bischoff et al. 2008; Mijnsbrugge et al. 2010; Vasques et al. 2012). Nonetheless, the role of seed source remains hard to predict, including because different species do not tend to respond in the same manner to similar conditions during seed development or following dispersal. For example, cold stratification has been found to either enhance or eliminate differences in germination between seed

sources, depending on the species studied (Milberg and Andersson 1998).

Seed dormancy together with seed size and seed dispersal mechanisms are key plant traits in determining where and when the seeds will germinate. Thus, these traits play an important role in regulating the spatio-temporal dynamics of vegetation community composition under a variety of changing environmental conditions (Venable and Brown 1988; Fenner and Thompson 2005).

The three species selected for this study—*Arbutus unedo* L., *Pistacia lentiscus* L. and *Phillyrea angustifolia* L.—are evergreen tall shrubs to small trees that often co-exist in the understory of forests dominated by *Quercus* spp. or *Pinus* spp. trees (Directiva Habitats 1992). All the three species have fleshy fruits that are dispersed by vertebrates (Herrera 1984, 1989) and none of them form a persistent seed bank (Cerabolini et al. 2003). They also have a low to moderate flammability, reflecting a limited accumulation of dead above-ground biomass (Liodakis and Kakardakis 2008) as well as a strong ability to resprout following damage, therefore contributing to ecosystem resilience (Vallejo et al. 2005; 2012).

Intensive disturbance regimes, possibly combined with unfavourable climate conditions during the initial recovery phase, are expected to endanger the persistence of the three species studied here (Pratt et al. 2013). Thus, in highly disturbed scenarios the lack of a persistent soil seed bank could per se justify the re-introduction of these species through ecological restoration.

An important criterion in selecting the three species studied here was their similar period of seed dispersal (autumn-winter), which conducts to the exposure to similar post-dispersal environmental conditions, i.e. cold and humid. The combination of high moisture and temperature conditions is known to favour seed aging, thus affecting negatively the seed vigour. Still, the exposure of imbibed seeds to cold conditions cold conditions in imbibed seeds has been found to induce the production of gibberellins, which, in turn, are known to promote germination in seeds with physiological dormancy (Penfield et al. 2005). Germination in seeds with physical dormancy is also influenced by temperature but not by moisture conditions, as the intact seed coat is impermeable to water entry (Long et al. 2014). Thus, cold but low moisture conditions are generally advised for ex situ conservation of orthodox seeds (Engelmann and Engels 2002).

A second key criterion in selecting the three species was that they represented the two principal types of dormancy: physical dormancy in the case of *P. angustifolia* (according to García-Fayos (2001)); physiological dormancy in the case of *A. unedo* (according to Tilki (2004)); and lack of dormancy in the case of *P. lentiscus* (according to García-Fayos and Verdu (1998) and García-Fayos (2001)).

Based on the species-specific dormancy types, we hypothesized that cold storage under high moisture conditions would: (i) enhance germination in *A. unedo*; (ii) either

increase or have no effect on germination in *P. angustifolia*; (iii) either have no effect or decrease germination in *P. lentiscus*. The effects of the treatments on *P. angustifolia* as well as on *P. lentiscus* were expected to depend on how susceptible the seeds would be to lose viability under high moisture conditions (Roberts 1973; Murdoch and Ellis 2000).

The overall aim of this study was to further the understanding of the germination ecology of three shrub species with special interest for ecological restoration of Mediterranean plant communities. Particular relevance was given to the role of different seed sources from distant regions in Portugal, which present contrasting climate conditions, also because seeds from regional sources are highly recommended in restoration actions to avoid outbreeding depression (Hufford et al. 2012). The study's specific objectives were the following: (i) to assess if the germination responses of the three selected species to cold storage would fit in with their contrasting types of dormancy; (ii) to determine if the germination responses of the three species varied between distant populations in Portugal with different temperature and rainfall regimes; (iii) to explore if possible differences in the germination response of distinct seed sources could be linked to differences in site-specific environmental conditions during the seeds' permanence in the soil seed bank in winter and, as such, could represent adaptations to local conditions.

2 Material and methods

2.1 Species distribution and sampling sites

Three evergreen Mediterranean shrub species—*P. lentiscus* (Anacardiaceae), *P. angustifolia* (Oleaceae) and *A. unedo* (Ericaceae)—were selected to represent different types of dormancy but comparable periods of seed dispersal (as detailed in the introduction).

P. lentiscus has a thermophilous geographical distribution pattern and is exclusive to the Mediterranean Basin and Macaronesia (Palacio et al. 2005; JBUTAD et al. 2014). In mainland Portugal, *P. lentiscus* occurs in approximately two thirds of the territory, and the northern population studied here is located at the north-western limit of the species' distribution (JBUTAD et al. 2014; Table 1). *P. angustifolia* has a distribution that is limited to the central and western parts of the Mediterranean Basin and that includes almost the entire mainland of Portugal, with the exception of some sparse areas (JBUTAD et al. 2014). *A. unedo* has the widest geographical distribution of the three species studied, occurring in Ireland, Southern Europe, Northern Africa, Palestina and Macaronesia. In mainland Portugal, *A. unedo* can be found throughout the entire territory (JBUTAD et al. 2014).

The geographical location, climate (long-term annual mean temperature and annual precipitation) and predominant soil

type of the sampling sites are given in Table 1. The populations that were sampled in this study differed noticeably in temperature but especially rainfall conditions, reflecting the strong climatic gradient that is present from northern to southern Portugal (Peel et al. 2007). Annual rainfall is, on average, approximately 20 to 40 % lower at the central than northern study sites and approximately 25 % lower at the southern than central sites (Table 1).

The exact locations of the sampled populations were selected by doing field surveys of the geographical areas that had been defined on beforehand. The surveys aimed at identifying populations with sufficiently large numbers of plant specimen presenting ripe fruits, which in practice, came to mean populations ranging from approximately 25 to more than 1,000 plants, within a target area of approximately 1 km². The sampling sites of *P. lentiscus* and *P. angustifolia* corresponded to shrublands that included individual trees but lacked a dominant tree layer, so that the sampled mother plants were small shrubs of less than 1.5 m high. These shrublands included, besides *P. lentiscus* and *P. angustifolia*, *Myrtus communis* L. and *Cistus* spp. as common elements, which, in its turn, were accompanied by *Crataegus monogyna* Jacq., *Ruscus aculeatus* L., *Quercus faginea* Lam. and *Laurus nobilis* L. at the northern sampling site, by *Olea europaea* L., *Daphne gnidium* L., and *Pinus pinea* L. at the central site, and by *Corema album* (L.) D.Don and *P. pinea* at the southern site. The sampling sites of *A. unedo* corresponded to *Pinus pinaster* Aiton plantations where it occurred in the understorey as tall shrubs 2–4 m high. Other companion species at these sites were *Erica* spp. and *Quercus* spp. in the northern location, *Calluna vulgaris* (L.) Hull, *Erica* spp., *Pterospartum tridentatum* (L.) Willk., and *Ulex* sp. in the central location, and *Cistus ladanifer* L., *Quercus suber* L. and *Ulex* sp. in the southern site.

2.2 Seed harvesting and handling

Seeds were harvested during the autumn of 2009, more precisely between November 12 and December 19. After identifying a target sampling area of approximately 1 km², ten mother plants were selected in a random manner but excluding plants that lacked sufficient ripe fruits, that showed apparent signs of damage or disease and that were located at less than 5 m distance from a previously selected plant. A suitable population of *P. angustifolia* could not be found in the previously-defined area in northern Portugal, in spite of an intensive survey campaign. Furthermore, the *P. angustifolia* populations selected at the central and southern sites lacked sufficient mother plants with ripe fruits, so that only five and nine plants were sampled, respectively. In the case of *P. lentiscus*, it was not possible to limit sampling to mother plants with sufficient black fruits, so that red fruits had to be harvested from various specimens. Based on the findings of

Table 1 Geographical coordinates, long-term annual mean temperature and annual precipitation (IPMA: data from 1981 to 2010, <https://www.ipma.pt/pt/clima/normais.clima/>) and principal soil type (Cardoso et al. 1973) for the different seed harvesting sites of *P. lentiscus*, *P. angustifolia* and *A. unedo*

Species	District	Site	Latitude	Longitude	Annual mean temperature (°C)	Annual precipitation (mm)	Principal soil type
<i>Pistacia lentiscus</i> and <i>Phillyrea angustifolia</i>	Aveiro	Northern	40°17'	8°90'	15.6	938	Cambisol
	Setúbal	Central	38°45'	9°06'	16.6	735	Luvisol
	Faro	Southern	37°19'	8°50'	17.9	509	Luvisol
<i>Arbutus unedo</i>	Braga	Northern	37°50'	8°40'	15.0	1,449	Cambisol
	Coimbra	Central	40°14'	7°99'	16.0	886	Cambisol
	Faro	Southern	31°14'	8°48'	17.9	509	Luvisol

Verdú and García-Fayos (1998; 2000), red fruits from *P. lentiscus* can be expected to contain higher percentages of abnormal seeds than black fruits. This possible bias was counterbalanced by excluding the floating fruits and seeds.

The harvested fruits were handled following a standardized protocol and for each individual mother plant separately. The first step of the protocol involved using a blender to separate the seeds from the fruit pulp. The seeds were then washed under running water and set to dry over filter paper for 2 weeks (García-Fayos 2001). The seeds were subsequently stored in hermetic glass bottles, using a layer of cotton and silica to guarantee low moisture conditions (6–8 %). The bottles were kept in the dark and at room temperature for 2–3 months until the start of the treatments. Before the actual start of the germination experiment, all seeds were subjected to a careful visual inspection and those with apparent signs of damage were excluded.

2.3 Treatments and experimental setup

Two cold storage treatments were selected to simulate conditions following seed dispersal in autumn-winter. These treatments consisted of storing the seeds in a cold chamber at 4–5 °C and in the dark for 10 weeks prior to the actual germination tests, either under low moisture conditions (LM) or under high moisture conditions (HM). The LM conditions involved maintaining the seeds in hermetic glass bottles (as described in the previous section), while the HM conditions involved adding vermiculite that was previously wetted to field capacity (free drainage was negligible) to the bottles and mixing it thoroughly with the seeds. The control treatment consisted of maintaining the seeds in the hermetic glass bottles at room temperature.

The germination tests comprised five replicates, each of which with up to 30 seeds. The seeds of each replicate consisted of three randomly selected seeds from each of the mother plants that, after being soaked for 24 h, were placed in a Petri dish using a regular grid. The Petri dishes were placed in a random scheme in a room with controlled conditions of temperature (20 °C) and photoperiod (16:8). Germination was

assessed at weekly intervals during a period of 14 weeks and was considered to have occurred when the radicle had emerged from the testa.

In the case of *P. angustifolia*, three further treatments were selected to specifically address the physical dormancy of the seeds (García-Fayos 2001; Piotto and Di Noi 2001). Two of them followed the same experimental setup as described above but involved an additional pre-treatment of the seeds: (i) heat exposure in a laboratory oven at 120 °C for 5 min, based on the findings of Salvador and Lloret (1995) for *Phillyrea latifolia* L. and (ii) mechanical scarification by removal of the seed coat, following García-Fayos (2001) and Piotto and Di Noi (2001). After a soaking period of 24 h, the seeds were set to germinate at 25 °C during the 14-week monitoring period. The third treatment involved a different experimental setup, with four replicates that each comprised 25 randomly selected seeds per mother-plant. The treatment itself involved the same pre-treatment of mechanical scarification as described before, which was then followed by overnight soaking and placing the seeds over moist filter paper. The germination conditions of this third additional treatment differed markedly from those of the other *P. angustifolia* experiments, since the seeds were placed in a germination chamber under dark conditions and at alternating temperatures of 10 and 20 °C (12 h), following García-Fayos (2001).

2.4 Data analysis

The cumulative germination percentages at the end of the 14 weeks monitoring period were first analysed by means of two-way ANOVA, using storage treatment and seed source as factors. In case these factors had a significant overall effect, the individual contrasts between treatments and/or seed sources were tested using Tukey's HDS post hoc test. The role of the treatments was also assessed for each seed source separately, using one-way ANOVA and Tukey's HDS post hoc test. The assumptions underlying ANOVA were assessed by visual inspection of the distribution of the residuals as well as by testing homoscedasticity by means of the Levene's test. In the case of *P. lentiscus* and *A. unedo*, the assumption of

homoscedasticity was met by transforming the germination percentages to $\ln(x+1)$ and $\sqrt{x}$, respectively. In the case of *P. angustifolia*, however, no standard transformation allowed to pass the Levene's test. Therefore, the overall importance of treatment and seed source in the germination percentages of *P. angustifolia* were tested separately, using the non-parametric Kruskal-Wallis test. The role of treatment was also tested for each of the individual *P. angustifolia* seed sources separately, using the Kruskal-Wallis test in combination with the Dunn's test for evaluating the different contrasts between treatments. The results of the statistical tests were considered significant at $\alpha \leq 0.05$. All statistical analyses were carried out using SPSS v.18.

3 Results

3.1 Overall effects of pre-germination treatments

In the control treatments, the three species differed noticeably in the time that they took to germinate (Fig. 1). The maximum germination percentages were attained much faster by the species lacking dormancy, *P. lentiscus*, than by the two species with physiological or physical dormancy, i.e. after 2–3 weeks in opposition to 12–14 weeks.

The three species also responded differently to the three storage treatments (Table 2). The final germination percentages of the species lacking dormancy, *P. lentiscus*, differed significantly among the three treatments, whereas the final germination of the two species with physical or physiological dormancy did not. In the case of *A. unedo*, the role of treatment depended in a significant manner on seed source, as revealed by the significant interaction term in the two-way ANOVA results (Table 2). In the case of *P. angustifolia*, the overall treatment effect was not significant, probably reflecting the poor germination observed in all replicate tests of the three common treatments. Germination of *P. angustifolia* was not substantially enhanced by either of the two additional treatments that involved germination under unvarying conditions of temperature. Final germination percentages remained below 5 % following the pre-treatments of both heat exposure and mechanical scarification. By contrast, the third additional treatment which combined mechanical scarification with alternating temperature conditions during germination resulted in final germination percentages of approximately 85 %.

The significant overall treatment effect on *P. lentiscus* germination reflected a negative effect of cold storage under high moisture conditions. The results of the Tukey post hoc tests showed that the final germination percentages were significantly lower (7 %) following cold storage under high moisture conditions than following cold storage under low moisture

conditions (18 %; $p < 0.01$) as well as under control conditions (13 %; $p < 0.05$).

3.2 Overall role of seed source

Seed source had a significant overall effect on final germination percentages in the case of *P. lentiscus* as well as in the case of *P. angustifolia* (Table 2), even though in the last species, a possible interaction with treatment effect could not be tested due to the lack of homoscedasticity (as detailed in 2.4). In the case of *A. unedo*, the role of seed source differed according to the treatments, as evidenced by the significant interaction between these two factors.

The significant overall effect of seed source on the germination of *P. lentiscus* coincided with significantly higher germination percentages for the seeds from the northern sampling (17 %) location than from the central location (7 %; $p < 0.01$), independent of the three common treatments (interaction: $p = 0.15$). There was furthermore some indication that the northern seeds of *P. lentiscus* also germinated better than its southern seeds. In the case of the HM treatment, the difference between the two seed sources was not only marginally significant ($p = 0.08$) but also noticeably (Fig. 1). In the case of *P. angustifolia*, the overall importance of seed source corresponded to significantly higher germination percentages for the southern (3 %) than central population (0 %). These differences were maintained following a successful protocol for germination enhancement (mechanical scarification plus germination at alternating temperatures), amounting 81 and 88 % for the central and southern seed source, respectively.

3.3 The effect of treatment within seed source

The final germination percentages of *P. lentiscus* differed in a consistent manner between the three storage treatments for the individual seed sources (Fig. 1). They increased from LM to control and to HM conditions. These differences between treatments appeared to increase along the geographical sampling gradient, from the northern to the central to the southern population (Fig. 1). This geographical pattern was also sustained by the one-way ANOVA results for the individual seed sources (Table 3). They revealed that the treatment-wise differences in final germination percentages were significant in the case of both the central population and the southern population, but not in the case of the northern population. The subsequent Tukey post hoc tests confirmed the overall tendency of a negative effect of cold storage under high as compared to under low moisture conditions (central and southern: $p < 0.05$).

In the case of *A. unedo*, the final germination percentages of the three common treatments only differed significantly for the seeds from the central population (Table 2). This significant difference corresponded to a positive effect of cold

Fig. 1 Cumulative germination percentages (average and standard error), over a period of 14 weeks, of seeds of three Mediterranean species with distinct types of dormancy (*P. lentiscus*, *A. unedo*, *P. angustifolia*) from

two to three contrasting sources in mainland Portugal (see Table 1) that were stored under standard conditions (control) as well as at low temperatures under low (LM) and high (HM) moisture conditions

storage under high moisture conditions (12 %) as compared to the control treatment (3 %; Tukey: $p=0.01$). However, such a

positive impact of cold storage under high moisture conditions was also suggested by various other instances, i.e.: (i) in the

Table 2 Statistical test results of the role of seed storage treatment and seed source in the germination percentages of *P. lentiscus* and *A. unedo* (using two-way ANOVA) as well as *P. angustifolia* (using Kruskal-Wallis test)

		Treatment	Seed source	Treatment $\times$ seed source
<i>Pistacia lentiscus</i>	<i>F</i>	11.26	7.1	1.83
	<i>p</i>	<0.01	<0.01	0.15
<i>Arbutus unedo</i>	<i>F</i>	6.13	81.79	3.02
	<i>p</i>	0.01	<0.01	0.03
<i>Phillyrea angustifolia</i>	QS	4.49	8.64	–
	<i>p</i>	0.11	<0.01	–

The treatments tested involved storage under standard conditions (control) as well as at cold temperatures under either low (LM) or high moisture (HM) conditions. Significant results (at $\alpha \leq 0.05$) were presented in *italics*

Table 3 Statistical test results for the role of seed storage treatment in the germination percentages of *P. lentiscus* and *A. unedo* (using one-way ANOVA) as well as *P. angustifolia* (using the Kruskal-Wallis test) for the 2–3 studied seed sources

		Treatment differences		
		Northern	Central	Southern
<i>Pistacia lentiscus</i>	<i>F</i>	1.76	4.59	5.64
	<i>p</i>	0.21	0.03	0.02
<i>Arbutus unedo</i>	<i>F</i>	3.00	6.28	0.75
	<i>p</i>	0.09	0.01	0.49
<i>Phillyrea angustifolia</i>	QS	–	0.00	6.71
	<i>p</i>	–	1.00	0.03

The treatments tested involved storage under standard conditions (control) as well as at cold temperatures under either control conditions (CT) and low (LM) or high moisture conditions (HM). Significant results (at $\alpha \leq 0.05$) were presented in *italics*

case of the central seed source, the lower germination percentages following cold storage under dry conditions; (ii) in the case of the northern seed source, the lower germination percentages of the other two treatments; (iii) in the case of the southern seed source, the slower initial germination response of the other two treatments during weeks 2 to 6.

In the case of *P. angustifolia*, none of the three common treatments resulted in the germination of even a single seed from the central population. However, they differ significantly in the final germination percentages of the southern seeds (Table 2). This significant difference corresponded to a negative impact of cold storage under high (0 %) as opposed to under low moisture conditions (6 %; Dunn's: $p < 0.05$).

4 Discussion

The present study found that seed storage conditions had distinct effects on the germination of three Mediterranean shrub species that had been selected to represent contrasting types of dormancy (physiological, physical, no dormancy) in autumn-winter dispersed seeds. Temporary storage under high-moisture cold conditions enhanced germination in *A. unedo*, had no effect on germination in *P. angustifolia* and reduced germination in *P. lentiscus*. These contrasting results agreed well with the hypotheses that were inferred from the species' dormancy types. A cold stimulus under high moisture conditions was hypothesized to break the physiological dormancy of *A. unedo* seeds, to be negligible for the physical dormancy in *P. angustifolia* seeds, and to lead to viability losses in the non-dormant seeds of *P. lentiscus*. In addition, the germination results that were obtained in the control treatment without temporary cold storage fitted in well with differences in type of dormancy. The seeds of *P. lentiscus* germinated faster than the seeds with—physical or physiological—dormancy, while the seeds of *P. angustifolia* germinated clearly less well than the non-dormant seeds or the seeds with physiological dormancy.

In general, the final germination percentages produced by the control treatment agreed well with the results reported by previous studies. In the case of *A. unedo*, most prior studies found very low germination percentages, varying between 0 and 5 % (Smiris et al. 2006: 0 %; Demirsoy et al. 2010: 1–3 %; Tilki 2004: 4 %; Ertekin and Kırdar 2010: 5 %). Hammami et al. (2005), however, obtained a considerably better result (19 %). In the case of *P. lentiscus*, the present results were of the same order of magnitude as the results reported by Quaoud (2007: 10 %) but they were considerably lower than the those obtained by Piotto and Di Noi (2001: 40–80 %). In the case of *P. angustifolia*, prior germination studies seemed to be lacking. Nonetheless, similar to this study, Takos and

Efthimiou (2003) obtained essentially no germination for *P. latifolia* seeds.

The positive effects of temporary cold storage on the germination of *A. unedo* reported here were in line with the findings of prior studies (Demirsoy et al. 2010; Ertekin and Kırdar 2010; Tilki 2004). The degree of germination enhancement in this study was of the same order of magnitude as that in Demirsoy et al. (2010: +3–20 %) but was markedly less than in Ertekin and Kırdar (2010: 50 %) and especially in Tilki (2004: 80 %). These discrepancies in germination enhancement could involve, among other factors, differences in the degree of dormancy that, in turn, could be related to the use of different seed sources. Even so, the maximum temperature threshold for germination in *A. unedo* seems to be relatively lower than the standard temperatures used in germination essays (20–25 °C). Markedly higher germination percentages (above 70–80 %) were observed in other studies using lower germination temperatures (10–15 °C; Bertsouklis and Papafotiou 2013; Vasques et al. 2013). This typical Mediterranean germination response (Fenner and Thompson 2005; Bertsouklis and Papafotiou 2013) explains the low germination percentages of *A. unedo* that were observed in this as well as in most of the prior studies (Tilki 2004; Smiris et al. 2006; Demirsoy et al. 2010; Ertekin and Kırdar 2010).

The degree of dormancy can influence the temperature thresholds for germination, with a decrease in dormancy broadening the range of temperatures that favour germination (Gutterman 2000). In turn, the degree of dormancy usually decreases in response to higher temperatures during seed development (Long et al. 2014). In the case of *A. unedo*, the observed increase in germination percentages along the sampling gradient from northern to southern Portugal agreed with a decrease in the degree of dormancy with increasing mean annual temperatures. The same tendency for higher germination in southern populations was also observed in *P. angustifolia*. Such a geographical pattern the degree of dormancy or sensitivity to dormancy breaking factors could represent an adaptation, by allowing *A. unedo* and *P. angustifolia* seeds to germinate earlier in regions where favourable temperatures occur earlier after seed dispersal.

The present finding that germination in *P. lentiscus* was negatively affected by cold storage under high moisture conditions contrasted with the results obtained by Quaoud (2007) as well as Piotto (1995). The former author observed a marked increase in germination (20 %), while the latter author reported a noticeable acceleration of germination in *P. lentiscus*. These contrasting results could be due to differences in the duration of the cold treatment, since the cold storage was more than twice as long in the present study (70 days) than in those of Quaoud (2007: 21 days) and Piotto (1995: 30 days). An increasing loss of seed viability with increasing storage period would agree with the finding of García-Fayos and Verdú (1998) that

P. lentiscus seeds exhibited a poor persistence in the soil seed bank.

Some of the differences in germination response between the seed sources could be interpreted as adaptive traits. For example, cold storage under high moisture conditions would not be expected to decrease germination in seeds from northern Portugal, since the region has a comparatively higher rainfall. By contrast, cold storage under high moisture conditions would be expected to decrease germination in seeds from a southern population, whereas cold storage under dry conditions would perhaps not.

The present findings seem relevant to the understanding of the ecology of Mediterranean plant communities that suffer frequent and/or severe disturbances, further aggravated by the foreseen climate changes of rising temperatures and more frequent and severe drought events (Solomon 2007). Germination on both *P. angustifolia* and *A. unedo* would be sensitive to changes in temperature regimes. In particular, germination in *A. unedo* could be hampered by a decline in cold and wet conditions during winter, which are required to reduce its degree of dormancy, as well as by an increase in temperatures during spring, which could inhibit germination. By contrast, various companion species of the studied species could be favoured by the foreseen climate changes, possibly leading to a shift in floristic composition. This is the case of various Mediterranean shrub species of the Cistaceae, Ericaceae and Leguminosae families and, in particular, those that form a permanent soil seed bank and whose dormancy is broken by dry and warm conditions (Vasques et al. 2012; Santana et al. 2013). Several of these species can markedly increase fuel load accumulation and therefore a shift in species composition could furthermore increase fire hazard, which in its turn could enhance ecosystem degradation (Pausas et al. 2012).

In this context, the storage of *P. lentiscus* seeds in the soil could be favoured by the foreseen drier and warmer winter conditions. Palacio et al. (2005) attributed the thermophilous distribution of *P. lentiscus* to the extended phenological activity of its canopy, which leads to the exposure of buds to cold winter. The present findings could, however, also contribute to explain the limits of distribution of this species since the observed low tolerance of the seeds to high moisture cold conditions could constrain the successful colonization of habitats with higher rainfall.

Finally, the observed differences in germination response among seed sets from different sources can improve the accuracy of high disturbance scenarios and/or climate change predictions by including the value of plant population adaptation. However, to fully understand the consequences of such site-related adaptations, their speed, degree and reversibility should be disentangled, namely by researching their origin and consequences under variable environmental conditions.

5 Conclusions

The present study allowed drawing five main conclusions on the germination ecology of three Mediterranean shrub species that were selected for their elevated potential for ecological restoration, on the one hand, and, on the other, for their contrasting dormancy types in their autumn-winter dispersed seeds. These five conclusions were the following: (i) *A. unedo*, *P. angustifolia* and *P. lentiscus* differed markedly in their germination responses to cold storage treatments simulating conditions in the soil seed bank following dispersal; (ii) the distinct responses, especially in terms of germination percentages, agreed well with the presence/absence and type of dormancy in these species, with cold storage under humid conditions enhancing germination in *A. unedo* (physiological dormancy), reducing it in *P. lentiscus* (no dormancy) and not affecting it in *P. angustifolia* (physical dormancy); (iii) seed source was a key factor in the germination response of all three species, either by having a significant overall effect or by having a significant effect that depended on the storage treatments; (iv) some of the seed source-related differences in germination behaviour could involve adaptive traits, as they seemed to fit well with the differences in climate conditions at the seed harvesting sites; (v) the role of mother plant life story as well as environmental conditions during seed development in seed dormancy degree or sensitivity to dormancy breaking factors require further research, including to improve protocols for seed harvesting, seed storage and germination tests in Mediterranean shrub species.

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