Research Paper

*Author for Correspondence:
A. Santiago,
E-mail: conservador@jardinbotanico-clm.com

CAMBRIDGE
UNIVERSITY PRESS

Seed dormancy break and germination by a rare relict of the Würmian glaciation in the Iberian Peninsula: *Euonymus latifolius* (Celastraceae)

A. Santiago[1,2]* [iD], J. M. Herranz[1,2] [iD] and P. Ferrandis[1,2] [iD]

[1]Botanic Garden of Castilla-La Mancha, Avda. de La Mancha s/n, Albacete 02006, Spain and [2]Botanic Institute of the University of Castilla-La Mancha, Avda. de La Mancha s/n, Albacete 02006, Spain

Abstract

Southern European peninsulas have repeatedly played notable roles as refuges in the natural history of flora during periods of glaciation. *Euonymus latifolius* (Celastraceae) is a relict species from the last Würmian glaciation in the Iberian Peninsula. It still lives with isolated populations in favourable, cool mountainous microhabitats, being an extremely rare, critically endangered species. These Iberian populations are often high-priority targets for conservation due to their long-term persistence and unique evolutionary trajectory. Previously, it has not been feasible to promote significant programmes for reintroduction and/or population reinforcement of this singular plant species due to the great difficulties of conventional propagation. In this study, we analysed the effects of temperature, light and gibberellic acid (GA$_3$) on the germination responses of *E. latifolius* to develop an effective protocol for seed germination as a main outcome. The results are coherent with the climatic temperature conditions recorded broadly in the Iberian Peninsula in the past and in the current refuge locations for the taxon. The germination responses of *E. latifolius* are compatible with those of seeds with intermediate physiological dormancy. In particular, the seeds required a 10-week warm period (20/7°C + 15/4°C) followed by 20 weeks of cold period (5 + 1.5°C) to break dormancy and achieve germination values over 90%. GA$_3$ also promoted germination (80%). Therefore, we developed the first effective protocol for promoting *E. latifolius* seed germination and, thus, sexual propagation, to facilitate urgent *ex situ* actions in the current climate change context.

Introduction

It is widely accepted that climatic changes in the Pleistocene were among the crucial determinants of the current distribution of plant species in temperate latitudes (Bennett, 1997). During the last Würmian glaciation, the Iberian Peninsula played a crucial role as a vast refuge for various types of biotas, and this was the source of most of the current Iberian flora (Hewitt, 1996; Taberlet et al., 1998; Vogel et al., 1999; Heredia et al., 2007). Species adapted to cold overcame glacial stages in peripheral refugia (Schonswetter et al., 2005). Some of the species that adapted best to cold probably extended their distribution ranges and/or latitudes during cold periods in warm interglacial or interstadial periods (Vargas, 2003). However, after the Würm glaciation period finished, the climate became warmer and drier over the Mediterranean basin (Blondel et al., 2010). Under these new environmental conditions, species that expanded their distribution range during cold periods became isolated at high altitudes or survived in refugia in the southern European peninsulas (Birks and Willis, 2008). Marked genetic isolation has been demonstrated for some Iberian plant lineages (Drovetski et al., 2018) because of their unique evolutionary trajectory, and thus plant populations in the Iberian Peninsula are high-priority targets for conservation (Rodriguez-Sanchez et al., 2010).

Euonymus latifolius (L.) Mill. (Celastraceae) is one of the species that has survived as a relict since the last ice age in the Iberian Peninsula. This deciduous, nemoral shrub or small tree species is distributed throughout Europe (up to the Caucasus), the Irano-Turanian region and North Africa (Middle Atlas, Tell Atlas and Kabylie) (Benedí, 1997; Gutiérrez et al., 2004). However, the Iberian populations are limited to a few refuges in favourable, cool mountainous microhabitats, which are mainly dispersed over the Sistema Ibérico Meridional mountain range (Gutiérrez et al., 2004; Peña et al., 2018), with very low numbers of individuals present in all cases (Gutiérrez et al., 2004; García and Sánchez, 2007). *E. latifolius* is considered the rarest small tree species in the Iberian Peninsula due to its markedly relict condition and low number of plants (López González, 2001). Indeed, in Spain, *E. latifolius* is designated critically endangered according to the IUCN criteria (i.e. CR B2ab(iii,iv) C2a(i); Moreno, 2011),

and it is classified with this threat category in the red book of Spanish flora (Gutiérrez et al., 2004).

Due to the extreme rarity and singularity of the Iberian relict *E. latifolius* populations, there is currently great interest in producing plants with conservation goals, and thus there is an urgent need to develop effective protocols for seed germination. However, little information is available regarding this key step in effective *ex situ* conservation programmes. Physiological seed dormancy has been found in the plant family Celastraceae (Baskin and Baskin, 2014), and previous tests demonstrated that conventional sexual propagation is very difficult for *E. latifolius* (Herrera et al., 1999; Gutiérrez et al., 2004). The low seed germinability in the Iberian populations may be related to seed dormancy mechanisms that require unknown environmental conditions, which could have been determined by their relict nature. This low seed germinability problem greatly hinders population reinforcement and it must be overcome to allow basic, urgent conservation actions for the relict Iberian lineage.

In this study, we aimed to overcome the difficulty of propagating *E. latifolius* from seeds and develop an effective *ex situ* sexual propagation protocol. This information is essential for implementing *in situ* population reinforcement programmes. In addition, we aimed to obtain valuable information regarding the conditions for natural plant recruitment and, thus, the viability of the relict populations. The specific goals of this study were to explore the germination ecology of *E. latifolius*, including determining whether it exhibits seed dormancy, and identify the factors and conditions that govern its germination patterns. Thus, we evaluated the effects of temperature, gibberellic acid (GA_3) and the light conditions on dormancy break and germination of seeds collected from relict natural populations.

Material and methods

Seed sources

Seeds were collected in the Sistema Ibérico Meridional mountain range from the core distribution area in the Iberian range of *E. latifolius*. In particular, seeds were collected from populations in Palomera (30TWK83) and Torcas de los Palancares (30SWK82), in Cuenca province (central–southern Spain). On 29 September 2021, we collected 500 apparently healthy and fully mature fruits. In the next 24 h, around 2300 apparently healthy, fresh seeds were extracted from the fruits. The seeds were washed to fully remove the remaining orange aril pulp and then treated with a solution of the fungicide benomyl ($500\,mg\,l^{-1}$). Germination tests were started immediately after cleaning the seeds.

General conditions for germination tests

Four replicates were tested for every germination treatment. In each replicate, 25 seeds were incubated in Petri dishes (diameter = 9 cm) on two layers of filter paper, which were kept saturated with distilled water. The dishes were sealed with Parafilm to minimize the loss of water and placed in germination chambers (model F-4; Ibercex, Madrid, Spain) equipped with digital temperature and light controls. We checked the dishes periodically to assess seed germination. Seeds were considered to have germinated when the radicle emerged. At the end of the experiment, the viability of non-germinated seeds was assessed based on tetrazolium tests (Cottrell, 1947). The germination percentages were

based on the number of viable seeds in our calculations, as recommended by Baskin and Baskin (2014). During periodical checks, we also watered the filter paper as necessary to maintain the humidity at an appropriate level. In addition, we inspected the dishes and if fungal contamination was observed, the seeds were immediately removed, washed carefully while avoiding vigorous mechanical friction, and the filter paper was replaced before returning the seeds to the dish. All germination tests started on 1 October 2021.

Effects of temperature conditions on germination

Since mature *E. latifolius* seeds have permeable coats and a fully developed embryo at the time of seed dispersal (personal observation), we ruled out both physical and morphological dormancy (*sensu* Baskin and Baskin, 2003). Therefore, we explored the possibility that seeds had physiological dormancy using a 'move-along' stratification thermal approach (*sensu* Baskin and Baskin, 2014). The design of the temperature sequences was based on the timing of seed dispersal by *E. latifolius*, that is, late September, and the temperature regime in the seed collection area, as follows: (1) fluctuating day/night temperatures of 20/7°C, which correspond to the mean maximum and minimum temperatures in October, respectively; (2) 15/4°C, which correspond to the mean maximum and minimum temperatures in November, respectively; and (3) a constant temperature of 5°C, which is close to the mean temperature during the winter months, combined with reductions to 1.5°C to simulate temperature decreases in the natural habitat. In total, we tested six temperature treatments and had three controls (Table 1). In the fluctuating temperature treatments, the germination chambers were programmed for thermoperiods of 24 h, where the maximum and minimum mean temperatures alternated every 12 h.

Effects of light conditions on germination

The 'move-along' thermal treatments described above were performed twice, with one complete set under photoperiod conditions and the other in full darkness. The photoperiod involved exposing seeds to light for 12 h and darkness for 12 h. In the fluctuating temperature treatments, the light phase was programmed to coincide with the highest temperature and the dark phase with the lowest temperature. This light–dark cycle (light treatment) was controlled using cold white fluorescent tubes ($25\,mol\,m^{-2}\,s^{-1}$; 1350 lx). Seeds incubated under dark conditions (darkness treatment) were wrapped in a double layer of aluminium foil to prevent the passage of light.

In tests that included photoperiod in the treatment, germination was checked every 2–3 days and germinated seeds (i.e. seeds with emergent radicles) were removed. In the darkness treatments, germination was checked once every 4 weeks under a dim green light (Vandelook et al., 2007) following the same criteria and procedure as indicated above.

Effects of GA_3 on germination

We prepared two sets of 16 seed replicates to determine whether GA_3 could substitute for the effect of thermal treatment on seeds in overcoming dormancy. In one set of Petri dishes, filter papers were moistened with a solution of 2000 ppm GA_3 throughout the test, whereas the other parallel set was moistened only with distilled water as a control (Santiago et al., 2013). Each replicate

Table 1. Thermal 'move-along' treatments tested in the study

Treatment	Temperature sequence	Total (weeks)
T1: Autumn → winter → spring	20/7°C (4 weeks) → 15/4°C (6 weeks) → 5°C (15 weeks) → 15/4°C (5 weeks)	30
T2: Long winter → cold spring	5°C (22 weeks) → 15/4°C (8 weeks)	30
T3: Long autumn → cold winter	20/7°C (4 weeks) → 15/4°C (6 weeks) → 5°C (2 weeks) → 1,5°C (11 weeks) → 5°C (7 weeks)	30
T4: Long autumn → cool winter	20/7°C (4 weeks) → 15/4°C (6 weeks) → 5°C (20 weeks)	30
T5: Short autumn → cold winter	15/4°C (4 weeks) → 5°C (4 weeks) → 1,5°C (15 weeks) → 5°C (1w)	24
T6: Short autumn → cool winter	15/4°C (4 weeks) → 5°C (20 weeks)	24
Control 5	5°C (30 weeks)	30
Control 15/4	15/4°C (30 weeks)	30
Control 20/7	20/7°C (30 weeks)	30

Temperatures (in °C) and lengths of thermal phases (in weeks) in each 'move-along' thermal treatment are shown.

set was treated as follows: four dishes were placed at 5°C (22 weeks) → 15/4°C (8 weeks) in light and another four received the same thermal treatment in darkness; and four dishes were placed at 15/4°C (16 weeks) in light and another four received the same thermal treatment in darkness. Germination was checked weekly until the first seed germinated and then at intervals of 2–3 days.

Statistical analysis

Germination was expressed as the final cumulative percentage of germinated seeds. Mean values and standard errors were calculated for germination percentages ($n = 4$). The effects of temperature, light and GA_3 on germination were determined by multifactorial analysis of variance (ANOVA). If the F-ratio obtained by ANOVA was significant, pairs of cases with differences were identified using Tukey's multiple comparison tests (1949). Before further analyses, the normality of data was checked using the David test (1954), and the homogeneity of variance was checked with the Cochran test (1941). Percentages were arcsine square-root-transformed, but the untransformed data are presented in the figure. In all tests, the significance level (α) was 5%.

Results

Effects of temperature and light conditions on germination

Only treatments that included a 10-week period of autumn temperatures (20/7°C → 15/4°C) followed by at least 15 weeks of cold stratification (5°C, or 5°C → 1.5°C; i.e. T1, T3 and T4) were highly effective in promoting germination (>50%; Table 2). Among these treatments, T3 was the most successful (germination > 90%), where cold stratification at 5°C alternated with an 11-week period of very cold stratification at 1.5°C, thereby simulating very harsh winter conditions. Treatment T5 also stimulated germination but in a less intense manner. Indeed, October temperatures (i.e. 20/7°C) were particularly effective in promoting seed germination (i.e. T3 *vs* T5; T1 *vs* T6; Table 2). In addition, a long period of cold stratification at 5°C was only effective when preceded by autumn temperatures (T4 and T6 compared with the control 5°C; Table 2). Different light conditions had no significant effects on germination (Table 2).

Germination rate

Germination was slow. The seeds required a long (around 25 weeks) initial stratification period at moderately warm

Table 2. Effects of thermal 'move-along' treatments, both in light and darkness, on the percentage of germination of *E. latifolius*

Treatments		% Germination	
		Light	Darkness
20/7°C (4 weeks) → 15/4°C (6 weeks) → **5°C** (15 weeks) → **15/4°C** (5 weeks)	T1	62 ± 1[Ea]	56 ± 5[Ea]
5°C (22 weeks) → 15/4°C (8 weeks)	T2	2 ± 0[Aa]	0 ± 0[Aa]
20/7°C (4 weeks) → 15/4°C (6 weeks) → 5°C (2 weeks) → 1,5°C (11 weeks) → **5°C** (7 weeks)	T3	94 ± 1[Ga]	90 ± 3[Ga]
20/7°C (4 weeks) → 15/4°C (6 weeks) → **5°C** (20 weeks)	T4	62 ± 0[Ea]	61 ± 2[Ea]
15/4°C (4 weeks) → 5°C (4 weeks) → 1,5°C (15 weeks) → **5°C** (1 weeks)	T5	48 ± 1[Da]	43 ± 3[Da]
15/4°C (4 weeks) → **5°C** (20 weeks)	T6	20 ± 0[Ca]	13 ± 5[Cb]
5°C (30 weeks)	Control 5°	4 ± 2[Ba]	6 ± 0[Ba]
15/4°C (30 weeks)	Control15/4°	0 ± 0[Aa]	0 ± 0[Aa]
20/7°C (30 weeks)	Control 20/7°	0 ± 0[Aa]	0 ± 0[Aa]

Upper-case letters show significant differences among records within columns and lower-case letters between pairs of cases in rows (significance level = 0.05). Within each thermal treatment significantly promoting germination, temperatures at which seeds germinated are indicated in bold.
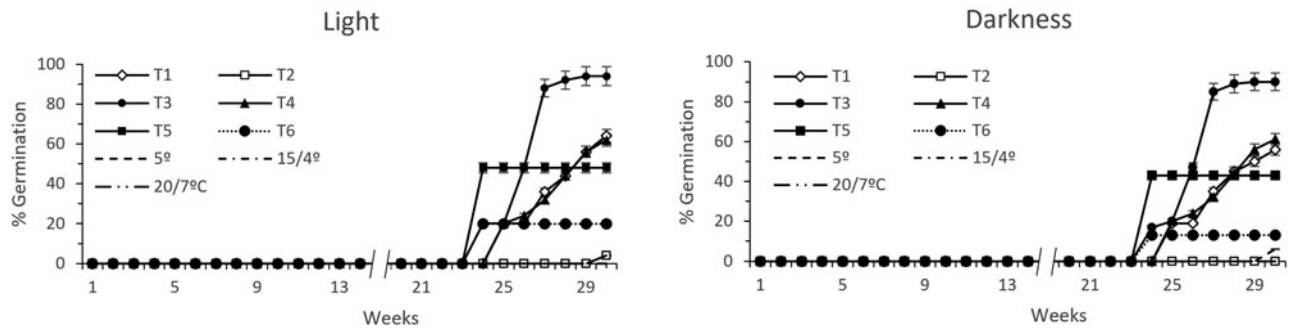
Figure 1. Sequence of the germination process grouping the light and dark tests. The figure shows the germination percentages obtained for each treatment represented with lines. The bars represent the maximum and minimum temperatures (dark grey and light grey, respectively).

temperatures followed by cold temperatures to initiate germination ([Fig. 1](#)). In the most effective treatment, that is, T3, half of the final cumulative germination, was reached 2 weeks after the first seed germinated (i.e. week 26 of testing), which was followed by a rapid increase to reach 90% in around 1 week. In the other effective treatments, that is, T1 and T4, the germination percentages increased gradually between weeks 25 and 30 ([Fig. 1](#)).

Effects of GA₃ on germination

GA$_3$ massively activated germination from null values in the controls to high percentages when seeds were treated with this phytohormone ([Table 3](#)).

Discussion

Mature *E. latifolius* seeds have permeable coats and a fully developed embryo at the time of seed dispersal and they required several months under favourable conditions to germinate, which overall points to the existence of physiological seed dormancy. In addition, GA$_3$ massively promoted germination in move-along thermal treatments with different temperature sequences. Thus, we conclude the seeds exhibited intermediate physiological dormancy. This germination pattern is very similar to that found in its congener *Euonymus europaeus* L., where germination occurs in two phases, with a requirement for moderate temperatures in the first phase (9–10°C) and cold stratification at 0–3°C for several weeks in the second phase. However, GA$_3$ is only weakly effective at promoting germination by *E. europaeus* seeds because it substitutes for the first phase of stratification but not the second phase

of stratification. Thus, *E. europaeus* seeds are considered to exhibit deep physiological dormancy (Nikolaeva et al., [1973](#)).

Variations in the germination requirements among species and populations of a single species may indicate adaptation to the local habitat conditions (Santiago et al., [2013](#)). Dormancy is often related to altitude and populations that experience prolonged snow cover require longer periods of cold stratification (Cavieres and Arroyo, [2000](#)). In *E. latifolius*, seed dormancy break requires exposure to moderately warm temperatures (20/7°C → 15/4°C) for 10 weeks, which are typical of the autumn months, followed by cold stratification (5 and/or 1.5°C) for 15 weeks. Subsequently, the seeds will germinate at 5 or 15/4°C, that is, typical temperatures in late winter–early spring. In the localities where our seeds were collected, seed maturation and dispersal occur at the end of September, thereby ensuring that seeds incorporated in the ground will be exposed to moderately warm temperatures during the following 2 months (20/7°C in October and 15/4°C in November) before the arrival of cold winter temperatures (5°C). Throughout the winter, the seeds may experience very cold temperatures (i.e. 1.5°C) during some weeks, and these temperatures clearly increased the germination percentages (T3) in the present study. This germination pattern reflects strong adaptation because the timing of seedling emergence in March will allow young plants to avoid the harshest frosts in winter and to experience 3–4 spring months of mild weather conditions when they can develop and acquire resources in order to successfully withstand the summer drought. This germination strategy is very common in species with intermediate or deep physiological dormancy (Baskin and Baskin, [2014](#)), as well as some species with non-deep complex morphophysiological dormancy, where the seeds become germinable after a sequence of moderately warm temperatures, followed by several months of cold stratification (e.g. *Narcissus longispathus*; Herranz et al., [2013](#)). Furthermore, these conditions corresponding to particularly long and cold winters may be similar to those typically found in mid-latitude mountain environments, such as Iberian ranges, during Quaternary cold stages, where the low temperatures favoured glacial expansion, whereas warm periods promoted periglacial dynamics (Oliva et al., [2019](#)).

Only the temperature determined the germination of *E. latifolius* seeds. Different light treatments (i.e. seed incubation in the photoperiod *vs* full darkness) had no significant effects on germination. Other light radiation treatments could be tested, but the fact that both extreme light conditions had no effects on germination by *E. latifolius* seeds was surprising due to the well-known nemoral and ombrophilous nature of this species (López

Table 3. GA₃ effects on the germination percentage of *E. latifolius*

Treatments		% Germination	
		Light	Darkness
5°C (22 weeks) → 15/4°C (8 weeks)	Control	2 ± 0^{Aa}	0 ± 0^{Aa}
5°C (22 weeks) → **15/4°C** (8 weeks)	GA$_3$	80 ± 1^{Ca}	79 ± 2^{Ca}
15/4°C (16 weeks)	Control	0 ± 0^{Aa}	0 ± 0^{Aa}
15/4°C (16 weeks)	GA$_3$	64 ± 2^{Ba}	62 ± 2^{Ba}

Upper-case letters show significant differences among records within columns and lower-case letters between pairs of cases in rows (significance level = 0.05). Within each thermal treatment significantly promoting germination, temperatures at which seeds germinated are indicated in bold.

González, 2001; Martín Herrero et al., 2003). During the early phases of recruitment, *E. latifolius* juveniles are particularly sensitive to light and they tend to concentrate (up to 80% of young individuals) under the tree canopy in sub-Mediterranean and Mediterranean mixed forests (Agea et al., 2021). Our more recent studies of *E. latifolius* recruitment have shown that the amount of photosynthetic active radiation (PAR) is a powerful bottleneck that affects the growth and survival of adult plants of this relict species (Santiago et al., in preparation). Plants died in short periods (less than 1 month) when directly exposed to visible light.

This apparent incongruity between the light requirements during germination and the juvenile and adult phases may be due to the genuine, natural conditions in the past nemoral habitat of *E. latifolius*, where attenuated illumination and shady conditions may be guaranteed, thereby making the light conditions irrelevant for the selective processes filtering the dormancy and germination mechanisms in this species. However, the current climate change scenario promoting particularly marked shifts in the environmental conditions over southern and central mountains in the Iberian Peninsula and affecting the structure and density of plant canopy (Sanz and Galán, 2020) may emerge as an acute risk for these residual populations, as their relict habitat transforms, and the species has no mechanism to prevent germination under stronger light conditions.

However, major increases in temperature (Nogués-Bravo et al., 2007; Sanz and Galán, 2020) above the thermal requirements for seed germination are even more concerning because they can greatly threaten *E. latifolius* recruitment in the Iberian Peninsula. The total number of *E. latifolius* plants censused in the Iberian Peninsula was 429 individuals, which were distributed in 15 populations over a relatively small biogeographic territory in three Spanish provinces comprising Cuenca, Jaén and Teruel (Pinedo, 2013). At present, these Iberian populations are adversely affected by (1) significantly decreasing humidity conditions in the habitat, which are critical for this genuinely nemoral taxon; (2) non-negligible herbivory pressure limiting natural plant regeneration in many populations and (3) intense competition from other plant species that are better adapted to the current climate conditions (Bañares et al., 2008). Thus, the presumed further restrictions on recruitment in the near future due to the loss of favourable thermal habitat conditions and strict species-specific temperature germination requirements, as well as other challenging factors, may lead to a dramatic change in the conservation status of *E. latifolius* in Iberia and even an extinction vortex scenario *sensu* Gilpin and Soulé (1986).

Several priorities must be urgently addressed to deal with this critical situation. In particular, genetic analyses are required to determine the degree of the singularity of the meridional European relict population genotypes to assess the actual conservation effort needed. *Chaerophyllum aureum* L. is another Iberian plant relic that shares habitat with *E. latifolius*, and recent studies have shown that the genotypes of this plant in Sistema Ibérico localities (central Spain) are contained in the genetic pool of northern European populations (Santiago et al., 2019). Thus, the genotypes of *C. aureum* in Sistema Ibérico localities are not as singular as originally suspected. In parallel and pending on the genetic analysis results, *ex situ* conservation actions could be performed. The results shown in the present study make the implementation of *E. latifolius* sexual propagation programmes feasible. Some considerations on seed sources, however, are needed. *E. latifolius* is a particularly scarce plant in southern Europe, so extreme caution should be paid if seeds are collected from natural, relict populations to avoid compromising the source of natural sexual regeneration (Baskin and Baskin, 2014). Germplasm banks can offer alternative seed sources. Indeed, seed accessions are currently stored in some Spanish germplasm banks (i.e. in Andalusia, Vadillo-Castril in the Cazorla mountain range and the Botanic Garden of Castilla-La Mancha; Gutiérrez et al., 2004; Herranz et al., 2011). However, *E. latifolius* seeds do not tolerate desiccation to low moisture contents, so they cannot be stored hermetically for a long term at cool near-zero or subzero temperatures (Holmes and Buszewicz, 1959; Rudolf, 1974; Hong et al., 1996). Therefore, seed availability in these institutions is temporally limited. Yet another option may be to conduct *ex situ* plant cultivation programmes, as implemented successfully in the plant collections at the Botanic Garden of Castilla-La Mancha (Santiago et al., in preparation), which could supply seeds for propagation if plantation size is large enough to guarantee certain genetic diversity.

Conclusions

In the current climate change context, *ex situ* actions and genetic analyses are urgently needed to design effective conservation programmes for the relict nemoral plant species *E. latifolius* in the Iberian Peninsula. Iberian populations of this species are particularly endangered because their germination ecology is adapted to past Würmian periglacial climate and habitat conditions, which are now being lost rapidly in this region. In addition, the populations are very small, and other threats exist, such as herbivory and competition. In this study, we developed an effective new protocol for promoting seed germination, and thus sexual propagation, for urgent *ex situ* actions, which is particularly suitable for restoration programmes, and pending genetic analyses should provide information regarding the singularity of the genotypes of this species present in the Iberian populations.

References

Agea D, García-de Lucas S and Lorite J (2021) Regeneration of submediterranean species *Euonymus latifolius* (L.) Mill. at its southernmost limit in Europe. *Mediterranean Botany* 42, e68137.

Bañares A, Blanca G, Güemes J, Moreno JC and Ortiz S (2008) *Lista roja 2008 de la flora vascular española. Dir. Gen. de Medio Natural y Política Forestal (Min. de Medio Ambiente, y Medio Rural y Marino).* Madrid, SEBICOP.

Baskin JM and Baskin CC (2003) Classification, biogeography, and phylogenetic relationships of seed dormancy, pp 518–544 *in* Smith RD, Dickie JB, Linington SH, Pritchard HW and Probert RJ (Eds), *Seed conservation: turning science into practice*, Kew, Royal Botanic Gardens.

Baskin CC and Baskin JM (2014) *Seeds. Ecology, biogeography, and evolution of dormancy and germination* (2nd edn). San Diego, Academic Press.

Benedí C (1997) *Euonymus* L., pp. 175–179 in Castroviejo S, et al (Eds) *Flora iberica, Plantas vasculares de la Península Ibérica e Islas Baleares 8*, Madrid, Real Jardín Botánico, C.S.I.C.

Bennett KD (1997) *Evolution and ecology: the pace of life.* Cambridge, Cambridge University Press.

Birks HJB and Willis KJ (2008) Alpines, trees, and refugia in Europe. *Plant Ecology Diversity* 1, 160.

Blondel J, Aronson J, Bodiou JY, Boeuf G and Fontaine C (2010) *The Mediterranean region: biological diversity in space and time.* Oxford and New York, Oxford University Press.

Cavieres LA and Arroyo MTK (2000) Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae) – altitudinal variation in the Mediterranean Andes of central Chile. *Plant Ecology* 149, 1–8.

Cottrell HJ (1947) Tetrazolium salt as a seed germination indicator. *Nature* 159, 748.

Drovetski SV, Fadeev IV, Raković M, Lopes RJ, Boano G, Pavia M, Koblik EA, Lohman YV, Redkin YA, Aghayan SA, Reis S, Drovetskaya SS and Voelker G (2018) A test of the European Pleistocene refugial paradigm, using a Western Palaearctic endemic bird species. *Proceedings: Biological Sciences* 285, 1–8.

García O and Sánchez I (2007) Nueva población de *Euonymus latifolius* (L.) Mill. (Celastraceae) en la Provincia de Cuenca. *Flora Montiberica* 37, 43–46.

Gilpin ME and Soulé ME (1986) Minimum viable populations: the processes of species extinctions, pp. 13/34 in Soulé ME (ed.), *Conservation biology: the science of scarcity and diversity.* Sinauer.

Gutiérrez L, Blanca G, Fabregat C, López S, Luque P, Benavente A, et al. (2004) *Euonymus latifolius* (L.) Miller, pp. 248–249 in Bañares A (Eds) *Atlas y libro rojo de la flora vascular amenazada de España, 2ª edición*, Madrid, Dirección General de Conservación de la Naturaleza.

Heredia H, Carrión JS, Jiménez P, Collada C and Gil L (2007) Molecular and palaeoecological evidence for multiple glacial refugia for evergreen oaks on the Iberian Peninsula. *Journal Biogeography* 34, 1505–1517.

Herranz JM, Martín J, Copete MA, Monreal JA and Ferrandis P (2011) Evaluación de riesgos de extinción y prioridades para la conservación de la flora silvestre y vegetación en Castilla-La Mancha, pp. 103–123 in Hernandez-Bermejo JE; Herranz-Sanz JM (Eds) *Protección de la diversidad vegetal y de los recursos fitogenéticos en Castilla-La Mancha: La perspectiva existente y el compromiso del Jardín Botánico*, Albacete, España, Instituto de Estudios Albacetenses 'Don Juan Manuel'.

Herranz JM, Copete E and Ferrandis P (2013) Non-deep complex morphophysiological dormancy in *Narcissus longispathus* (Amaryllidaceae): implications for evolution of dormancy levels within section *Pseudonarcissi*. *Seed Science Research* 23, 141–155.

Herrera CM, Molero J, Muñoz J and Valdés B (1999) *Libro Rojo de la Flora Silvestre Amenazada de Andalucía*, pp. 135–137. *Tomo I: Especies en Peligro de Extinción*. Sevilla, Consejería de Medio Ambiente, Junta de Andalucía.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* 58, 247–276.

Holmes GD and Buszewicz G (1959) *Forest tree seed investigations.* London, Report on Forest Research, Forestry Commission.

Hong TD, Linington S and Ellis RH (1996) *Seed storage behavior: a compendium.* Rome, International Plant Genetic Resources Institute.

López González G (2001) *Guía de los árboles y arbustos de la Península Ibérica y Baleares (especies silvestres y las cultivadas más comunes).* España, Ediciones Mundi-Prensa.

Martín Herrero J, Cirujano S, Moreno M, Peris JB and Stübing G (2003) *La vegetación protegida en Castilla-La Mancha.* Madrid, Junta de Comunidades de Castilla-La Mancha.

Moreno JC (2011) *Lista Roja de la Flora Vascular Española 2010. Actualización con los datos del Adenda 2010 al Atlas y Libro Rojo de la Flora Vascular Amenazada.* Madrid, Dirección General de Conservación de la Naturaleza y Sociedad Española de Biología de la Conservación de Plantas.

Nikolaeva MG, Daletzkaya TV, Razumova MV and Kofanova NN (1973) Effects of gibberellin and kinetin on embryo growth and seed germination in spindle tree and Tatar maple. *Soviet Plant Physiology* 20, 600–605.

Nogués-Bravo D, Araújo MB, Errea MP and Martínez-Rica JP (2007) Exposure of global mountain systems to climate warming during the 21st century. *Global Environmental Change* 17, 420–428.

Oliva M, Palacios D, Fernández-Fernández JM, Rodríguez-Rodríguez L, García-Ruiz JM, Andrés N, Carrasco RM, Pedraza J, Pérez-Alberti A, Valcárcel M and Hughes PD (2019) Late quaternary glacial phases in the Iberian Peninsula. *Earth-Science* 192, 564–600.

Peña A, Feliu JF, Lozano JL and García O (2018) Contribución al conocimiento de la distribución y demografía de *Euonymus latifolius* (l.) Mill. (Celastraceae) en el sistema ibérico. *Flora Montiberica* 72, 8–16.

Pinedo S (2013) *Población, amenazas y distribución de Euonymus latifolius (L.) miller (Celastraceae) en la península Ibérica.* Murcia, VI Congress of Plant Conservation Biology.

Rodríguez-Sánchez F, Hampe A, Jordano P and Arroyo J (2010) Past tree range dynamics in the Iberian Peninsula inferred through phylogeography and palaeodistribution modelling: A review. *Review of Palaeobotany and Palynology* 162, 507–521.

Rudolf PO (1974) *Euonymus* L., pp. 393–397 in Schopmeyer CS (Eds) *Seeds of woody plants in the United States. Agriculture Handbook No. 450*, Washington DC, Forest Service, USDA.

Santiago A, Herranz JM, Copete E and Ferrandis P (2013) Species-specific environmental requirements to break seed dormancy: implications for selection of regeneration niches in three *Lonicera* (Caprifoliaceae) species. *Botany* 91, 225–233.

Santiago A, Ahrazem O, Gómez-Gómez L, Copete MA, Herranz R and Ferrandis P (2019) Climate-mediated selection, deep complex morphophysiological dormancy, embryo growth, interpopulation variability. *Turkish Journal of Botany* 43, 6.

Sanz MJ and Galán E (2020) *Impactos y riesgos derivados del cambio climático en España. Oficina Española de Cambio Climático.* Madrid, Ministerio para la Transición Ecológica y el Reto Demográfico.

Schönswetter P, Stehlik I, Holderegger R and Tribsch A (2005) Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molecular Ecology* 14, 3547–3555.

Taberlet P, Fumagalli L, Wust-Saucy AG and Cosson JF (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7, 453–464.

Vandelook F, Bolle N and van Assche JA (2007) Multiple environmental signals required for embryo growth and germination of seeds of *Selinum carvifolia* (L.) L. and *Angelica sylvestris* L. (Apiaceae). *Seed Science Research* 17, 283–291.

Vargas P (2003) Molecular evidence for multiple diversification patterns of alpine plants in Mediterranean Europe. *Taxon* 52, 463–476.

Vogel JC, Rumsey FJ, Schneller JJ, Barrett JA and Gibby M (1999) Where are the glacial refugia in Europe? Evidence from pteridophytes. *Biological Journal of the Linnean Society* 66, 23–37.