



Seed germination of *Phillyrea angustifolia* L., a species of difficult propagation

Sara Mira, Alberto Arnal and Félix Pérez-García

Departamento de Biotecnología-Biología Vegetal, E.T.S. de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain

Abstract

Aim of study: The purpose was to determine the type of dormancy and the optimal germination conditions of *Phillyrea angustifolia* (Oleaceae) seeds.

Area of study: Germination requirements of *P. angustifolia* seeds collected from wild plants growing in the province of Ávila (Central Spain) were studied.

Materials and methods: Seed water uptake was measured. Seeds with and without an endocarp were germinated at different temperatures, and several treatments were tested.

Main results: The lignified endocarp interferes mechanically with the emergence of the radicle, and the treatments that achieved the highest germination percentages were the total removal of the endocarp with pliers (84%) or the immersion in liquid nitrogen for 1 min (97%). Scarification with concentrated sulphuric acid did not significantly increase germination compared to the control seeds, and treatments with dry heat or wet heat were detrimental to seed germination. The optimum temperature for germination was 15 °C. A pre-sowing treatment of soaking in distilled water for 24 h slightly increased germination speed. Neither cold stratification at 5 °C nor soaking in a gibberellic acid solution improved seed germination.

Research highlights: *Phillyrea angustifolia* seeds have physiological dormancy – that is, the embryo does not have enough growth potential to overcome the mechanical restriction of the lignified endocarp. The seeds do not exhibit physical dormancy, given their water-permeable lignified endocarp. Our results suggest that the optimum germination protocol for *P. angustifolia* would be the total removal of the endocarp or immersion in liquid nitrogen for 1 min, followed by immersion in distilled water for 24 h and then seed incubation at 15 °C in light or darkness.

Keywords: Oleaceae; physical dormancy; physiological dormancy; seed scarification; stony endocarp.

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Correspondence should be addressed to Félix Pérez-García: felix.perez@upm.es

Introduction

Plants living in Mediterranean ecosystems face serious environmental constraints. These plants cope with extreme summer droughts and frequent fires that play an important role in such ecosystems (Chaves *et al.*, 2002). Climate change might alter the hydrological cycle in the Mediterranean region (Mariotti *et al.*, 2008), and models predict a reduction in total precipitation and drier summers (Christensen *et al.*, 2007) as well as an increase in fire hazard (Moriondo *et al.*, 2006; Pausas *et al.*, 2008; Moreno *et al.*, 2010). In Mediterranean areas, drought, fire and plant regeneration strategies are closely interlinked (Parra *et al.*, 2012; Orsenigo *et al.*, 2014;

Keyes & Manso González, 2015); therefore, knowledge of seed germination of Mediterranean species is a key factor for ecosystem conservation and for directing regeneration efforts (Thomas & Garcia-Marti, 2015).

Phillyrea angustifolia L. (Oleaceae), narrow-leaved mock privet or evergreen privet, is a small tree that appears scattered in dense evergreen Mediterranean forests and tall shrublands. It grows in well-preserved habitats across warm and dry areas and plays an important role in post-fire ecological dynamics (Herrera *et al.*, 1994; Vitale *et al.*, 2007). Many of the natural and semi-natural forests in which *P. angustifolia* grows are under decline due to climate change and centuries of

intense land use (Blondel *et al.*, 2010), and restoration efforts are underway (Gómez-Aparicio *et al.*, 2004). Moreover, this thermophilic and low-water-demanding species is increasingly used for landscaping purposes (De Marco *et al.*, 2005). In recent years, there has been a renewed interest in *P. angustifolia* plant production for use in general planting and restoration programmes.

Phillyrea angustifolia can be propagated by seed as well as vegetatively, but growth of cuttings is difficult (Piotto & Di Noi, 2003). Both in nature and in nurseries, poor, unreliable seed germination is common (Catalán, 1991; Traveset *et al.*, 2007). The genus *Phillyrea* produces blue-black drupes, usually containing a single seed, which are dispersed by animals, such as goats and birds (Herrera *et al.*, 1998; Traveset *et al.*, 2007; Andrés, 2011). Due to their lignified endocarp, it has been suggested that *Phillyrea* seeds might have physical dormancy (García-Fayos *et al.*, 2001; Takos & Efthimiou, 2003). However, water absorption by the endocarp has not been determined, and dormancy-breaking protocols have not been optimised (Catalán, 1991). Better knowledge of the germination behaviour of *P. angustifolia* seeds is crucial for the establishment of an efficient propagation protocol.

The study reported here provides evidence of the effects of different environmental conditions and treatments on the germination of *P. angustifolia* seeds. The specific objectives of the study were: (1) to elucidate the kind of dormancy; (2) to establish an optimised seed scarification treatment; and (3) to investigate the optimal conditions for the seed germination and seedling emergence of *P. angustifolia*.

Materials and methods

Seed collection

Mature fruits of *P. angustifolia* were collected in September 2012 from a wild population located in the province of Ávila (central Spain) and provided by Semillas Montaraz S.A. The fleshy exocarp and mesocarp were manually removed at the laboratory (Figure 1). Seeds with an endocarp were stored for one month under laboratory conditions (at ~23 °C, under darkness, 35% relative humidity) until the start of trials, in October 2012. Initial seed viability was evaluated by a tetrazolium test. Seeds were cut in half and submerged in a 1% solution of tetrazolium chloride for 24 h in darkness at 25 °C. Initial seed viability of the lot was 88%.

Water uptake during seed imbibition

To determine the water uptake capacity during seed imbibition, four replicates of 10 seeds (with and without

an endocarp) were weighed and then placed into Petri dishes on filter paper moistened with distilled water. After 1, 2, 4, 6, 10, 24, 48, 72 and 168 h of imbibition, seeds were quickly surface-dried with filter paper and then reweighed. Percentage of water uptake (mean \pm standard error) was calculated as the amount of water absorbed by seeds relative to the initial seed mass.

Germination assays

For all germination trials, four replicates of 25 seeds were incubated in 9-cm-diameter glass Petri dishes, on two sheets of filter paper moistened with 4 mL of distilled water. Seeds were selected randomly within the seed lot, which consisted of 500 g of seeds. In order to avoid contamination, seeds were disinfected previously in 10% hydrochloric acid for 5 min and then washed with distilled water for 50 s. Filter papers were rewetted regularly with distilled water, as required. Samples were checked daily, and germinated seeds were counted and removed. The criterion of germination was normal seedling development (ISTA, 2009). The incubation period was 100 days. Seeds that had not germinated at the end of the incubation period were cut opened and, if empty, excluded from analyses. The number of empty seeds was always \leq 2% of the total number of seeds.

Seed scarification treatments

Different scarification treatments were applied to seeds with an endocarp before incubation at 15 °C with a 16-h light photoperiod:

- Mechanical scarification: endocarp was totally removed using pliers.
- Dry heat: seeds were placed in an oven at 50 °C, 80 °C or 100 °C for 30 min.
- Wet heat: seeds were immersed in distilled water at 80 °C for 5 min or at 100 °C for 5 sec and then allowed to cool in the same water at room temperature for 2 h.
- Sulphuric acid (H₂SO₄): seeds were immersed in sulphuric acid (96%) for 1 min and then repeatedly washed with distilled water before sowing.



Figure 1. Fruit and seed of *Phillyrea angustifolia*.

- Liquid nitrogen (LN): seeds were immersed in LN (-196 °C) for 1 min, 30 min, 1 h or 24 h before sowing.

Germination temperature and light regimes

Seeds with and without an endocarp were tested for germination at different constant temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C) and alternate temperature regimes (20/10 °C and 25/15 °C) with a 16-h light photoperiod provided by cool white fluorescent tubes with an irradiance of 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For alternating temperature regimes, the higher temperature was programmed for 16 h in light and the lower one for 8 h in darkness. Also, seeds were incubated under total darkness at 15 °C.

Pre-sowing treatments

Seeds without an endocarp were incubated at 15 °C with a 16-h light photoperiod after different pre-sowing treatments:

- Distilled water: Seeds were soaked in distilled water at room temperature (~23 °C) for 24 h.
- Gibberellic acid (GA_3): Seeds were soaked in a GA_3 solution (1000 mg L^{-1}) at room temperature (~23 °C) for 24 h.
- Cold stratification: Seeds were stored in moist vermiculite under darkness at 5 °C for 30, 60 or 90 days.

Statistical analysis

The statistical analysis of seed germination data was performed using the approach proposed by Ritz *et al.* (2013) with the package ‘drc’ (Ritz & Streibig, 2005) of the software environment R (R Core Team, 2015). We used a nonlinear log-logistic model to relate the cumulative germination and to monitor time after initialisation of the test [1].

$$F(t) = d / (1 + \exp[b(\log(t) - \log(MGT))])$$

where d is the maximum germination percentage; MGT (mean germination time) is the time where 50% of the seeds that germinated during the experiment have germinated; and b is proportional to the slope of F at time t . The estimation of nonlinear regression parameters was based on treating the data as event time – that is, considering the monitoring interval during which seeds germinated or the time interval of the entire experiment if they did not germinate. Thus, we have a multinomial distribution across these intervals, and this distribution was used to obtain the parameter estimates by maximum likelihood. The time–event model implemented in the

‘drc’ package allows parameter comparison among germination curves for different treatment groups.

Results

Water uptake during seed imbibition

Seeds of *P. angustifolia* without an endocarp imbibed water quickly after 24 h, and seed mass increased by 47% (Figure 2). Seeds with an endocarp absorbed water at a lower rate, and, after 24 h, their mass had increased by 25%. After 72 h, the mass of seeds with and without an endocarp had increased by 39% and 53%, respectively. After 7 days, the mass of seeds without an endocarp was greater (54%) than that of seeds with an endocarp (41%). Results of water uptake in seeds with and without an endocarp are expressed as percentage of initial mass in order to not account for differences in mass between samples due to the mass of the endocarp itself.

Seed scarification treatments

Germination data were fitted to a nonlinear log-logistic model curve, and values of maximum germination and MGT were calculated. Control seeds of *P. angustifolia* with an endocarp showed slow germination (68 day MGT), with maximum germination increasing progressively up to 59% (Table 1, Figure 3). On the other hand, seeds whose endocarp had been removed mechanically achieved 84% of germination with a MGT of 22 days. Also, immersion in LN for 1 min achieved high germination (97%), albeit slowly (70 day MGT) (Table 1). Seeds treated with H_2SO_4 showed a significantly lower germination

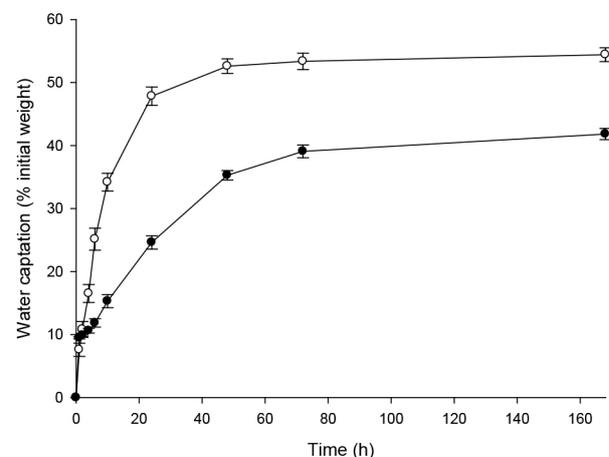


Figure 2. Water hydration curve (% initial weight) of *Phillyrea angustifolia* seeds with an endocarp (●) and without an endocarp (○).

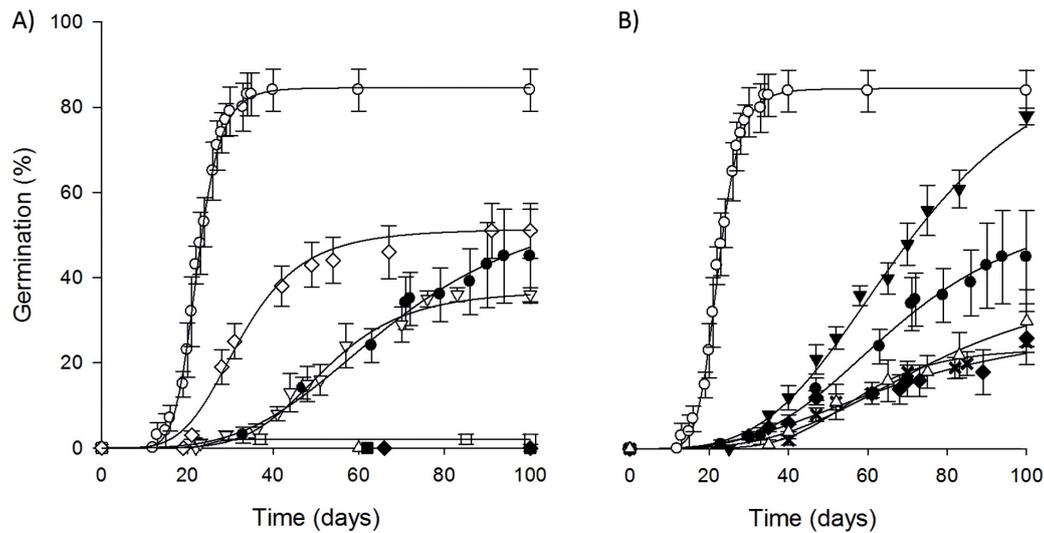


Figure 3. Germination time courses of *Phillyrea angustifolia* seeds with an endocarp after different treatments. Warm and acid treatments (A): control with endocarp (●); manual scarification (○); dry heat at 50 °C (▽), at 80 °C (△) and at 100 °C (■); wet heat at 80 °C (□) and at 100 °C (◆); and sulphuric acid for 1 min (◇). Liquid nitrogen scarification (B): control with endocarp (●); manual scarification (○); and liquid nitrogen for 1 min (▽), 30 min (△), 1 h (×) and 24 h (◆). Values are averages of four replicates ± standard error. Data were fitted to a nonlinear log-logistic model (curves), and values of maximum germination and mean germination time were calculated.

Table 1. Seed germination curve parameters of *Phillyrea angustifolia* seeds with an endocarp after different pre-sowing treatments: maximum germination percentage (mean ± standard error (SE)) and mean germination time (MGT ± SE). Parameters were estimated by maximum likelihood from a nonlinear log-logistic model with a multinomial distribution. Within a column, values followed by the same letters are not significantly different ($p > 0.05$). Mean germination time was not calculated (NC) when final germination was equal to or less than 5%.

Treatment	Germination (% ± SE)	MGT (days ± SE)
Non-treated seeds (control)	59 ± 11 bc	68 ± 9 de
Mechanical scarification	84 ± 4 a	22 ± 1 a
Dry heat		
50 °C	37 ± 5 bed	52 ± 3 db
80 °C	0	NC
100 °C	0	NC
Wet heat		
80 °C	2 ± 1 e	NC
100 °C	0	NC
Sulphuric acid (1 min)	51 ± 5 b	32 ± 2 c
Liquid nitrogen (-196 °C)		
1 min	97 ± 3 a	70 ± 6 e
30 min	40 ± 11 bcd	76 ± 14 e
1 h	24 ± 4 d	57 ± 4 bde
24 h	29 ± 8 cd	66 ± 16 bde

percentage (51%) than those whose endocarp had been removed mechanically (84%). Seeds treated with LN for 30 min or more germinated less than non-treated seeds. Likewise, neither dry nor wet heat significantly improved seed germination.

Germination temperature and light regimes

Significant differences ($p < 0.05$) of final germination percentages and germination speed were observed among germination incubation temperatures of seeds with and without an endocarp (Table 2, Figure 4). Germination percentages reached by *P. angustifolia* seeds with an endocarp were 45–53% at temperatures ≥ 15 °C, with slow germination (MGT values ranged from 54–77 days) (Figure 4). Germination of seeds without an endocarp ranged from 83–90% for any temperature between 15 °C and 25 °C. Moreover, 15 °C presented the best results in terms of germination speed, with slight differences between light and darkness (Table 2).

Pre-sowing treatments

The effect of different pre-sowing treatments on the germination of seeds without an endocarp is shown in Table 3 and Figure 5. No significant differences ($p > 0.05$) were found among the final germination percentages reached by control seeds (non-treated seeds without an endocarp) nor seeds soaked in distilled water. However, both GA₃ and stratification at 5 °C for 30–90 days significantly decreased the final

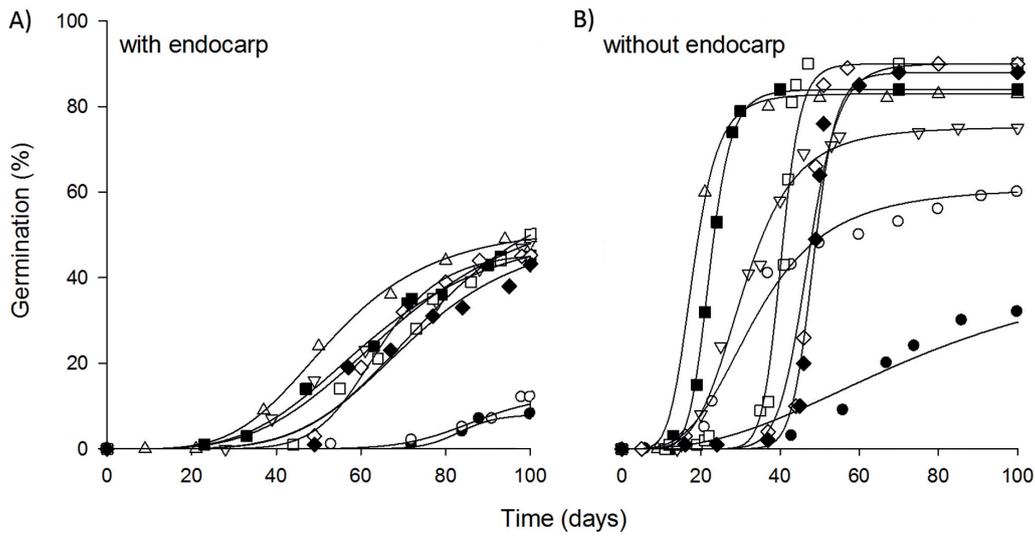


Figure 4. Germination time courses of *Phillyrea angustifolia* seeds with an endocarp (A) and without an endocarp (B) at different temperatures and light conditions: 5 °C (●), 10 °C (○), 10/20 °C (▽), 15 °C darkness (Δ), 15 °C (■), 20 °C (□), 25 °C (◇) and 25/15 °C (◆). Values are averages of four replicates. Data were fitted to a nonlinear log-logistic model (curves), and values of maximum germination and mean germination time were calculated.

germination percentage compared to that of the control ($\leq 42\%$ vs. 84% , respectively). Cold stratification at 5 °C was also detrimental to seeds with an endocarp (Table 3). Germination speed was faster in seeds soaked in distilled water than in the other treatments.

Discussion

Information on the germination strategies of Mediterranean plant species is most relevant for

ecosystem conservation, especially in the context of climate change. Our data provide useful information on germination protocols for ex situ propagation of *P. angustifolia*. The regeneration potential of a given species depends on the effect of ambient conditions on its propagation strategy, with high germination percentages and short germination times being desirable. In most conditions studied here, *P. angustifolia* seed germination was low, delayed and gradual, resulting in plants at different ages, which is undesirable from a commercial seedling production viewpoint.

Table 2. Seed germination curve parameters at different temperature regimes and light conditions of *Phillyrea angustifolia* seeds with and without an endocarp: maximum germination percentage (mean \pm standard error (SE)) and mean germination time (MGT \pm SE). Parameters were estimated by maximum likelihood from a nonlinear log-logistic model with a multinomial distribution. Within a column, values followed by the same letters are not significantly different ($p > 0.05$). Within a row, for each parameter (germination and MGT) and germination condition, the significance level between seeds with and without an endocarp is shown (p).

Temp. (°C)	Light conditions	Germination (% \pm SE)			MGT (days \pm SE)		
		With endocarp	Without endocarp	p	With endocarp	Without endocarp	p
5	light	8 \pm 3 b	45 \pm 15 c	*	83 \pm 3 c	78 \pm 24 cde	ns
10	light	13 \pm 3 b	61 \pm 5 c	***	87 \pm 5 c	34 \pm 2 c	***
15	light	52 \pm 7 a	84 \pm 4 ab	***	63 \pm 6 ab	22 \pm 1 b	***
15	darkness	52 \pm 6 a	83 \pm 4 ab	***	54 \pm 4 a	18 \pm 1 a	***
20	light	63 \pm 10 a	90 \pm 3 a	*	77 \pm 7 bc	40 \pm 1 d	***
20/10	light	60 \pm 10 a	75 \pm 4 bc	ns	70 \pm 8 abc	31 \pm 1 c	***
25	light	46 \pm 5 a	90 \pm 3 a	***	64 \pm 2 b	47 \pm 1 e	***
25/15	light	51 \pm 8 a	88 \pm 3 a	***	72 \pm 6 bc	48 \pm 1 e	***

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant.

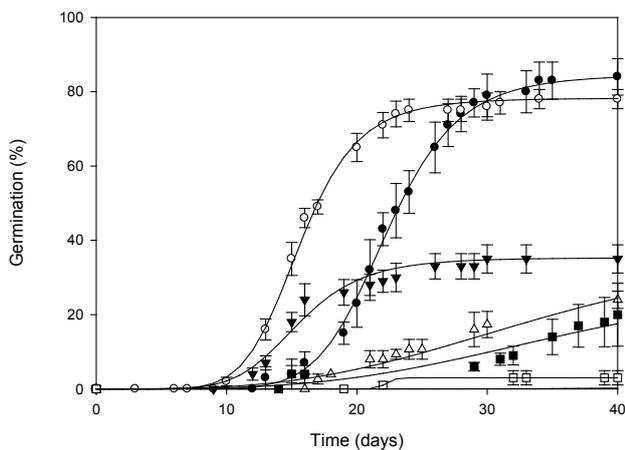


Figure 5. Germination time courses of *Phillyrea angustifolia* seeds without an endocarp after different pre-sowing treatments: control (●); distilled water (○); gibberellic acid (GA₃) (▼); and cold stratification at 5 °C for 1 month (△), 3 months (■) and 6 months (□). Values are averages of four replicates ± standard error. Data were fitted to a nonlinear log-logistic model (curves), and values of maximum germination and mean germination time were calculated.

The endocarp clearly impeded seed germination, with only half the seeds germinating after 100 days of incubation. A high and rapid germination was achieved after total removal of the endocarp with pliers, with most seeds germinating after 30 days. However, this technique is enormously time-consuming and highly dependent on operator experience, since the seed can be easily smashed. A high germination percentage also was achieved when seeds with an endocarp were scarified with a 1-min immersion in LN. Seeds germinated

gradually during a longer time period but with the advantage that this treatment can be standardised easily. Immersion in LN for longer time periods (30 min, 1 h, 24 h) resulted in lower germination percentages. Regarding the effects of temperature, the highest germination percentage was obtained at 15 °C. These results are in agreement with those of Thanos *et al.* (1992, 1995), who found that the optimal germination temperature for most Mediterranean shrub species ranges between 15 °C and 20 °C, and with previous data on *P. angustifolia* seeds collected at a different geographical location (Mira *et al.*, 2015b).

Common germination treatments generally recommended for forestry species did not lead to satisfactory results with *P. angustifolia*. Acid scarification has been suggested as the best technique to promote germination in several species with a stony endocarp (Young & Young, 1992), and this technique has been recommended previously for *Phillyrea* species (Bacchetta *et al.*, 2008; Ballesteros *et al.*, 2015), alone or followed by wet heat (Semillas Silvestres S.L., 2010). However, our results indicated that a 1-min immersion in H₂SO₄ achieved lower germination percentages than scarification with pliers or LN. While longer treatments obtained high germination amounts in previous studies (Bacchetta *et al.*, 2008; Semillas Silvestres S.L., 2010; Ballesteros *et al.*, 2015), results also indicate that a 30-min or 6-h treatment of H₂SO₄ was undesirable for seed germination in a different population of *P. angustifolia* (Mira *et al.*, 2015b). Our data also showed that cold stratification (5 °C) was detrimental to seed germination, either with or without an endocarp. Since longer cold stratification times reduced seed germination

Table 3. Seed germination parameters after different pre-sowing treatments for *Phillyrea angustifolia* seeds with and without an endocarp: maximum germination percentage (mean ± standard error (SE)) and mean germination time (MGT ± SE). Parameters were estimated by maximum likelihood from a nonlinear log-logistic model with a multinomial distribution. Mean values followed by the same letters within a column are not significantly different ($p > 0.05$). Mean germination time was not calculated (NC) when the final germination was equal to or less than 5%.

Treatment	Endocarp	Germination (% ± SE)	MGT (days ± SE)
Control (mechanical scarification)	Without	84 ± 4 a	22 ± 1 b
Soaking in distilled water (24 h)	Without	78 ± 4 a	16 ± 0 a
Soaking in GA3 (1000 mg L ⁻¹ ; 24 h)	Without	35 ± 5 bc	16 ± 1 a
Cold stratification (5 °C)			
30 d	Without	42 ± 8 bc	36 ± 5 c
60 d	Without	33 ± 6 cd	38 ± 4 c
90 d	Without	3 ± 2 e	NC
30 d	With	51 ± 6 b	58 ± 5 d
60 d	With	19 ± 5 d	78 ± 7 e
90 d	With	0	NC

even though seeds that did not germinate were not dead, as seen by the cutting test, we hypothesise that low temperatures induce secondary dormancy. Cold stratification is a commonly applied treatment for forestry species growing in areas with cold winters, and several seed companies recommend cold stratification for *Phillyrea* seed germination (Semillas Silvestres S.L., 2010; Vilmorin, 2013). The inefficacy of cold stratification detected in our results agrees with previous works on *Phillyrea* species (García-Fayos *et al.*, 2001; Mira *et al.*, 2015b), but it is nonetheless striking, since the *P. angustifolia* population studied here grows in an area with cold winters (mean temperature of the coolest month is -0.7°C).

Our results suggest that the lignified endocarp of *P. angustifolia* seeds interferes mechanically with the emergence of the radicle but not with the absorption of water. Albeit more slowly, seeds with an endocarp did imbibe water during soaking. This would indicate that, contrary to previous suggestions (García-Fayos *et al.*, 2001; Takos & Efthimiou, 2003), *P. angustifolia* seeds do not exhibit physical dormancy, which is defined as the presence of a water-impermeable layer in the seed or fruit (Baskin & Baskin, 2004). Since embryos are fully developed and seeds with and without an endocarp are water permeable, we conclude that *P. angustifolia* seeds have physiological dormancy (Baskin & Baskin, 2004) – that is, the embryo does not have enough growth potential to overcome the mechanical restriction of the lignified endocarp. Similar results were reported in several species with a lignified endocarp (Baskin *et al.*, 2002), including some species in the Oleaceae, such as *Olea* spp. (Cuneo *et al.*, 2010). In nature, *Phillyrea* fruits are ripe in September–October and are dispersed from September to March, and germination and seedling emergence take place from February to April (Herrera *et al.*, 1994; Andrés, 2011). Therefore, the endocarp might deteriorate in the soil during the season, allowing the seeds to germinate with early spring temperatures. Other authors found that seeds of *Phillyrea latifolia* are an important component of the soil seed bank (Ne'eman & Izhaki, 1999) and that they remain viable in the soil for more than 1 year (Herrera *et al.*, 1994; Yucedag & Gultekin, 2011). Others reported that most seedlings emerge from fruits produced during the previous reproductive event (Lloret *et al.*, 2004). Also, *Olea europaea* seeds do not germinate in the soil until the endocarp has been decomposed (Cuneo *et al.*, 2010). These observations suggest that warm followed by cold stratification might result in the germination of the species that is delayed and gradual during a period of time. By removing the endocarp or with LN scarification, a homogeneous and rapid germination can be achieved.

In accordance with the finding that the *P. angustifolia* endocarp is permeable, our results also have shown that scarification with H_2SO_4 was detrimental to seed viability. Acid scarification treatments are intended to mimic processes occurring when seeds are eaten and dispersed by animals. In field experiments, few seeds were recovered after being eaten by animals (Grande *et al.*, 2013), supporting our finding of a deleterious effect of acid. However, while seed germination increased when eaten by goats (Grande *et al.*, 2013), as seen in our data when compared to seeds with an endocarp, germination decreased when eaten by birds (Traveset *et al.*, 2008). These apparently contradictory results may be explained on the basis of inter-population variability in endocarp permeability and hardness, as previously suggested for other Mediterranean species (Correia *et al.*, 2014). A variation in seed germination requirements among populations also could explain the great differences found in germination response to temperature when compared to previous works. In our assay, germination at $20/10^{\circ}\text{C}$ was 75%, while previous assays with *P. angustifolia* seeds collected in a different Spanish population obtained values of 10% germination at $20/7^{\circ}\text{C}$ (Mira *et al.*, 2015b) or values of 90% at $20/10^{\circ}\text{C}$ or $20/7^{\circ}\text{C}$ (García-Fayos *et al.*, 2001; Herranz *et al.*, 2006). Intraspecific variation in seed response to GA3 also could explain that while our data show that GA3 is detrimental to seed germination, previous results indicated that GA3 allowed high germination (Mira *et al.*, 2015b). Intraspecific variation has been interpreted as one of the most important survival strategies for species growing under variable and unpredictable environmental conditions, as in Mediterranean forests, either in germination requirements (Kigel, 1995; Baskin & Baskin, 2014), seed dormancy (Pérez-García *et al.*, 2012; Copete *et al.*, 2014) or seed longevity (Lazar *et al.*, 2014; Mira *et al.*, 2011a, 2011b, 2015a).

Conclusions

Our results indicate that in *P. angustifolia*, the endocarp is water-permeable but may interfere mechanically with the emergence of the radicle. Total removal of the endocarp or immersion in LN for 1 min were the treatments that showed the best results. Optimal germination temperature for *P. angustifolia* seeds was 15°C , and germination speed was increased by pre-soaking in distilled water. Sulphuric acid slightly increased seed germination and germination speed. Dry or wet heat; cold stratification; and GA3 were not beneficial to seed germination. Our study emphasises the need for a species-by-species investigation when trying to establish the optimal germination protocol of

a species as well as suggesting the necessity of taking into account the possible intraspecific variation in germination requirements for *P. angustifolia*.

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