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Delayed effect of thermal treatment on breaking physical seed dormancy: intrapopulation variation and implications for soil seed banks

Miguel A. Copete Carreño[A,B,*], Elena Copete Carreño[A,B], Pablo Ferrandis Gotor[A,B] and Jose M. Herranz Sanz[A,B]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Miguel A. Copete Carreño
Department of Plant Production and Agricultural Technology, University of Castilla-La Mancha, Higher Technical School of Agronomic and Forestry Engineering and Biotechnology, University Campus s/n, Albacete 02071, Spain
Email: miguel.copete@uclm.es

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ABSTRACT

Background. Many studies have focused on the dormancy-breaking response to heat treatment of freshly matured seeds and immediately after thermal shock. **Aims.** We evaluated whether the full effect of dry heat scarification in freshly matured seeds could be delayed over time and the possible influence of previous storage in the soil. **Methods.** *Adenocarpus argyrophyllus* was the model species selected to explore our hypotheses by analysing the: (a) influence of scarification treatments; (b) seedling emergence during 5 years after dry heat scarification of freshly matured seeds, and evaluating intrapopulation variation; (c) seedling emergence after dry heat scarification of seeds rescued from soil; and (d) ability to form persistent soil seed banks. **Key results.** Dry heat scarification of freshly matured seeds only resulted in 22.5% germination. However, exposure to pre-sowing thermal shock stimulated seedling emergence during the first few years post-planting, with high intrapopulation variation. In seeds recovered from soil, thermal shock before reseeding increased the seedling emergence rate. **Conclusions and implications.** Our results show that, to avoid incomplete interpretation, studies of thermal treatment on the breaking of physical seed dormancy should allow the seeds sufficient time to exhibit the complete effects of high temperature treatment, thereby preventing underestimation.

Keywords: *Adenocarpus*, fire-prone ecosystems, germination, hard-seededness, heat scarification, Leguminosae, Mediterranean climate, post-fire regeneration, seedling emergence, soil seed bank.

Introduction

The dynamics of Mediterranean-type ecosystems reflect long-term adaptations to climatic stress and fire, and many plant species have developed responses that allow them to persist (Odion and Davis 2000; Ooi *et al.* 2014). In fire-prone ecosystems, post-fire resprouting and post-fire seeding are two independent traits, and species that live in these ecosystems may have one of the two traits (obligate resprouters or obligate seeders), both, or none (Pausas *et al.* 2004). In these ecosystems, seedling recruitment often occurs from a previously dormant soil seed bank, and fire seems to play a crucial role in stimulating germination (Keeley *et al.* 2011). Thus, the community that appears after a fire typically has a similar composition to that found previously as a widespread autosuccesion syndrome (Minnich and Bahre 1995; Ferrandis *et al.* 1999*a*).

If adult plants are capable of resprouting from belowground vegetative buds, seedling recruitment may not be needed to maintain a community after a single fire. However, mortality of some adult plants may occur during a fire and some senescence in inter-fire periods. Thus, some successful seedling recruitment will be necessary, with a greater proportion when aridity is greater (Pausas and Keeley 2014), in order to compensate for adult plant losses after a long series of fires (Auld and O'Connell 1991). Seedlings may also take advantage of the gap created by mortality of adult plants, where the higher levels of light, water and soil nutrients can promote their establishment (Liao *et al.* 2015).

Moreover, it should be noted that sexual reproduction may not be the most important quantitative mode of regeneration, but it can be significant because it is the only way of conserving the genetic variation in species that regenerate by resprouting (Tarrega *et al.* 1992). Therefore, it is also essential to identify the factors that affect the germination of seeds produced by resprouters because understanding the mechanisms of persistence under fire regimes is very important for interpreting the past and predicting future changes (Pausas and Keeley 2014). In the present study, we analysed the germinative ecology of the Mediterranean shrub known as 'cenizo', *Adenocarpus argyrophyllus* (Rivas God.) Caball. (Leguminosae).

The soil seed bank is formed of all viable seeds on the ground or buried in the soil. Persistent seed banks allow the re-establishment and maintenance of populations after disturbances without the need for an external seed source, and they can play important roles in years with poor seed set (Thompson *et al.* 1997; Copete *et al.* 2015). Massive post-fire seed germination has been recorded for many species in various Mediterranean ecosystems (Ferrandis *et al.* 1999*b*; Magaña Ugarte *et al.* 2021), thereby demonstrating the important roles of seed banks in secondary succession initiated after fires in these communities. Similarly, in species under some degree of threat, such as that analysed in the present study, the persistence of seeds in the soil ensures the maintenance of a population in an area, and this is a crucial mode of survival for threatened annuals such as *Sisymbrium cavanillesianum* Castrov. and Valdés Berm. (Herranz *et al.* 2003*a*) and biennials such as *Coincya rupestris* subsp. *rupestris* Porta and Rigo ex Rouy (Herranz *et al.* 2003*b*), even in years with no yield of seeds.

Hard-seededness, or physical dormancy, is a very common type of seed dormancy imposed by the presence of a hard testa, especially in species from the families Cistaceae and Leguminosae (Baskin and Baskin 2014). Seeds with physical dormancy are typically very long-lived (>100 years) when stored under dry, cool conditions (Fenner 1995) and they represent an important part of persistent soil banks (Ferrandis *et al.* 1999*b*; Holmes and Newton 2004). The temperatures experienced by soil seed banks during fires determine whether buried seeds survive and germinate after fires, and complex mosaics of spots exposed to a broad range of temperatures have been recorded (Auld and O'Connell 1991; Odion and Davis 2000). In leguminous species, heat breaks the innate seed dormancy by disruption of the seed coat, thereby allowing subsequent imbibition and germination (Gama-Arachchige *et al.* 2013) and several studies indicate that the high temperatures (50–150°C) generated in the superficial layers of soil promote germination (Tarrega *et al.* 1992; Herranz *et al.* 1998). Thus, legumes are considered important components of post-fire successional communities in Mediterranean climate regions (Huerta *et al.* 2022).

Adenocarpus argyrophyllus is an evergreen shrub endemic to the central–western part of the Iberian Peninsula, where it lives between 800 and 1800 m above sea level (asl) on siliceous substrates forming part of communities dominated by *Quercus* sp. or in scrubland plant communities (Castroviejo 1999). The freshly matured seeds do not imbibe water and they have hard coats, thereby indicating the presence of physical dormancy (Baskin and Baskin 2014). The communities inhabited by *A. argyrophyllus* have experienced great anthropic pressure for centuries, including forest fires, grazing and felling (López *et al.* 2019). Post-fire regeneration mainly occurs via basal resprouting but seedlings are also found in burnt areas (JM Herranz, pers. obs.).

Previous studies suggest that the effects of thermal shock on breaking physical dormancy could be deferred over time (e.g. Ferrandis *et al.* (1999*a*) found a large and mostly physically dormant *Cistus salviifolius* L. seed bank in the upper natural soil layer a few days after fire, but it was fully depleted 2 years later). In addition to the heat from fires, environmental factors related to the softening of hard-coated seeds include high summer temperatures, temperature fluctuations, alternate soaking and drying, freezing and thawing (Baskin and Baskin 1989; Ferrandis *et al.* 1999*a*). Thus, because of the above-mentioned delayed effects, most of the studies that conducted evaluations immediately after applying the shock (Rivas *et al.* 2006; Jastrzębowski *et al.* 2017; Daibes *et al.* 2019) may have underestimated the effects of high temperatures on some species.

Moreover, it is important to evaluate the effects of thermal shocks on seeds produced by different individuals because differential germination heat requirements for individuals could have adaptive value due to this mechanism ensuring post-fire germination and recruitment under a wide range of fire intensities and temperatures. Indeed, Liyanage and Ooi (2015) demonstrated that dormancy-breaking responses to heat treatments varied significantly among individual plants in five Australian shrub legumes. However, the present study is the first to attempt to monitor the responses of individual plant over time.

In particular, the objectives of this study were: (i) to investigate whether thermal shocks applied to seeds with physical dormancy could promote delayed release from dormancy over time, and whether the extent of this germinative response was affected by intrapopulation variability; (ii) to compare the sensitivity of freshly matured seeds and seeds from the soil bank to thermal shocks; and (iii) determine the implications for fire-prone population dynamics based on the existence of physically dormant seed banks in soil.

Methodology

Plant material and seed source

Adenocarpus argyrophyllus is a shrub that frequently reaches 4 m in height and individuals can produce more than 1000 seed pods with abundant glandular hairs. The seed pods are

4–6 cm in length and they contain 3–7 large seeds (~40–45 mg each), which are ovoid, laterally compressed and dark green. Ripening occurs at the end of July and the seed pods have an explosive opening mechanism, which allows the seeds to disperse up to 10 m away (JM Herranz, pers. obs.). These dense and streamlined seeds generate momentum in flight, which helps to maximise the explosive distance (Stamp and Lucas 1983). After dehiscence, many fruits still retain 1–2 seeds in their basal part and their stickiness allows them to adhere to the hairs of mammals, which actively contributes to their dispersal (Castroviejo 1999).

The study population was located in the Sierra de San Vicente (Universal Transverse Mercator UTM: 30TUK5346, 1240 m asl) facing north, with a quartzite and siliceous sand substratum, and it occupied an area of approximately 2 ha with an estimated population size of 190–230 adult individuals. The population was located in a mixed forest stand of *Quercus pyrenaica* Willd. and *Q. ilex* L. subsp. *ballota* (Desf.) Samp., with gaps occupied by broom shrubland containing *A. argyrophyllus*, *Genista florida* L., *G. cinerascens* Lange, *Cytisus scoparius* (L.) Link., *Pistacia terebinthus* L. and *Daphne gnidium* L. The area has a Mediterranean–continental climate with a summer drought and cold winters. Wildfires occur in this area with an average recurrence of 10 years (INFOCAM 2020).

On 26 July 2017, when dehiscence of the seed pods had already commenced, ~3000 seeds (from 750 seed pods) were randomly collected from 20 plants with a good crown conformation and vigorous and healthy appearance, and were stored in a paper bag. For trials to analyse intrapopulation variability, 10 additional healthy fruiting plants were selected and 300–350 seed pods were collected separately from each to obtain cohorts of 1200–1400 seeds, which were stored in separate paper bags. The seed pods exploded in the bags 0–2 days after harvesting to release the seeds, which were separated from the walls of the seed pods using a sieve with a mesh size of 0.5 cm. After cleaning, the seeds were stored in paper envelopes under laboratory conditions (22°C, 50% relative humidity).

Incubation conditions

Seed germination was tested in chambers equipped with a digital temperature and light control system (±0.1°C, cool white fluorescent light, 25 μmol m^{-2} s^{-1} (1350 lux)). Incubation was conducted under photoperiod conditions comprising 12 h light at 20°C and 12 h darkness at 7°C. The thermoperiod of 20–7°C was selected because it corresponds to October, which is the month when seedling recruitment mostly occurs (Benito 2013). The seeds were placed on two layers of filter paper saturated with distilled water in plastic Petri dishes with a diameter of 9 cm. Dishes were sealed with Parafilm to minimise the loss of water.

Radicle emergence (radicle ≥1 mm) was used as the criterion for determining whether germination occurred (Baskin and Baskin 2014). Seed germination was checked every 2–3 days for a period of 60 days and germinated seeds were removed. Non-germinated imbibed seeds with soft, yellowish cotyledons were considered non-viable. For the seeds still retaining physical dormancy, the testa was fractured and then the test period was extended until they germinated (viable) or did not and deteriorated (non-viable).

Two parameters were analysed after each germination test comprising the final cumulative percentage germination and the germination rate expressed as the T_{50} parameter, which is defined as the time required for half the final germination level (Thanos and Georghiou 1988).

Germination with scarification treatments

Four replicates of 50 seeds were used for each treatment starting on 1 September 2017.

- A set of 200 untreated seeds was used as the control.
- Another set of seeds was subjected to mechanical scarification by abrasion between two pieces of sandpaper (Tarrega *et al.* 1992; Kimura and Islam 2012) as a reliable reference to compare the effectiveness of thermal treatment in promoting seed germination (Herranz *et al.* 1998).
- An additional scarification treatment with hot water was tested. Seeds were immersed in a glass containing 100 mL of hot distilled water (100°C) and then left to cool to room temperature for 4 h, before placing the seeds in Petri dishes. This scarification technique has been used in previous studies to remove hard-seededness in legumes (Cushwa *et al.* 1968; Herranz *et al.* 1998).
- The set of scarification trials also included a dry heat treatment. Seeds were heated to temperatures similar to those recorded in the upper layer of the soil (0–10 cm depth) during forest fires in the garrigue in southern France (Trabaud 1979), Mediterranean-type ecosystems in southeastern Australia (Bradstock and Auld 1995) and Chile (Gómez-González and Cavieres 2009), i.e. 90°C for 10 min (90°C/10 min) and 120°C for 5 min (120°C/5 min). These temperatures have been used in similar previous studies of legume seed germination (Auld and O'Connell 1991; Herranz *et al.* 1998). Preheating was conducted by spreading dry seeds on glass dishes and heating in a muffle furnace (target temperature ±2°C).
- Two treatments combining dry heat and mechanical scarification were performed in order to evaluate whether thermal shock led to the loss of seed viability.

Cumulative seedling emergence after dry heat scarification of freshly matured seeds: intrapopulation variation

Ten seed cohorts were tested in this experiment, which started on 1 August 2017. Three sets each containing 300 seeds were prepared from each cohort, where one set was used as a control, one set was exposed to 90°C/10 min, and the other was exposed to 120°C/5 min. Three replicates each containing 100 seeds were prepared from each set. Each replicate was then sown in a PVC pot with a diameter of 20 cm and depth of 18 cm, which was filled with sand, where the seeds were placed approximately equidistant at a depth of 0.5–1 cm. The resulting 90 pots (10 cohorts × 3 seed sets × 3 replicates) were placed in an unheated, metal-framed shade house (690 m asl). Temperatures were recorded hourly using a data logger. To simulate humidity conditions in the natural habitat, pots were watered up to field capacity once each week, except in July and August when watering was reduced to once each fortnight. In addition, watering was withheld when the substratum was frozen in winter. Seedling emergence was monitored for 5 years until 1 December 2022, by scoring and removing emerged seedlings every 15 days. The seeds that did not germinate were recovered on 1 December 2022. The upper layer of sand was removed from each pot to a depth of 3 cm and the seeds were readily separated by washing through a 2 mm mesh sieve. The recovered seeds were classified as non-imbibed or imbibed seeds. The non-imbibed seeds were mechanically scarified and then incubated. The imbibed seeds were directly incubated. After this period, germinated seeds were considered viable and non-germinated seeds were non-viable.

Many seeds decomposed during the 5 years of the experiment. These seeds were considered non-viable when calculating the viability of the seeds in each replicate.

Cumulative seedling emergence after dry heat scarification of seeds recovered from soil

This experiment evaluated whether the hard-coated seeds might exhibit some softening of their coats when buried in the soil to check if this made the subsequent thermal shock more effective at promoting germination.

The trial began on 1 August 2017, using 1200 seeds from the population sample divided into 12 lots each of 100 seeds. Each lot was sown in a pot with sand as in the previous experiment and irrigation was applied at the same frequency. The seedlings were removed as they emerged. On 25 September 2019, the seeds were recovered as described for the previous experiment to obtain a total of 640 non-imbibed seeds with healthy appearance. One set of 200 seeds was exposed to 90°C/10 min and another set of 200 seeds to 120°C/5 min. A control treatment (no exposure to dry heat) was also conducted with another set of 200 seeds. From each of these three sets, four replicates each of 50 seeds were sown in pots with sand on 1 October 2019, as described for the previous experiment, before monitoring, scoring and removing seedlings that emerged until 1 July 2020. Seeds that did not produce seedlings were then recovered and categories were established as described for the previous experiment.

Soil seed bank

Soil seed bank sampling was conducted on 2 July 2017, approximately 3 weeks before seed dispersal began and after seedling emergence. The sampling season was determined based on Thompson and Grime's (1979) concept of a persistent soil seed bank. Sampling was conducted in 20 randomly located plots measuring 1 m^2. A cylindrical metal probe with an internal diameter of 4.5 cm was used to extract soil samples from two depth strata: stratum I at 0–5 cm and stratum II at 5–10 cm. Ten subsamples of stratum I extracts were collected from each plot and placed together in a plastic bag to form one sample, and the same procedure was conducted for stratum II.

In the laboratory, each sample was washed through a 2 mm-mesh sieve under a stream of running water. The large size of the *Adenocarpus* seeds facilitated their extraction using the physical separation method, where broken seeds and those with small holes or signs of predation were discarded. In order to differentiate between viable and non-viable seeds, they were mechanically scarified with sandpaper and subsequently incubated for 60 days, when germinated seeds were considered viable.

Statistical analysis

For germination tests, differences in germination percentages among scarification treatments were tested using generalised linear models (GLMs) with a binomial error structure and a logit link function to compare final germination and viability averages, followed by Tukey's test when differences were significant. The variable T_{50} was analysed in a similar manner but using a Poisson error structure.

For intrapopulation variation tests, GLMs with a binomial error structure and a logit link function were used to compare final germination percentages of individuals for each treatment separately. When differences between final germination percentages among individuals were significant, Tukey's test was used as the contrast method to compare their means. The same analyses were applied to seed viability for each individual.

All graphs were plotted using untransformed data. All statistical analyses were performed with SPSS software version 28 (IBM 2021).

Results

Germination of freshly matured scarified seeds

The germinability of untreated seeds (control) was very low (i.e. 3%) and it took 38 days for germination to start. By contrast, mechanical scarification of the seed coat resulted in very high (94%) and rapid (T_{50} = 13 days) germination (Fig. 1). Hot water scarification and dry heat scarification also increased final germination, but to a lesser extent. The combination of dry heat followed by mechanical

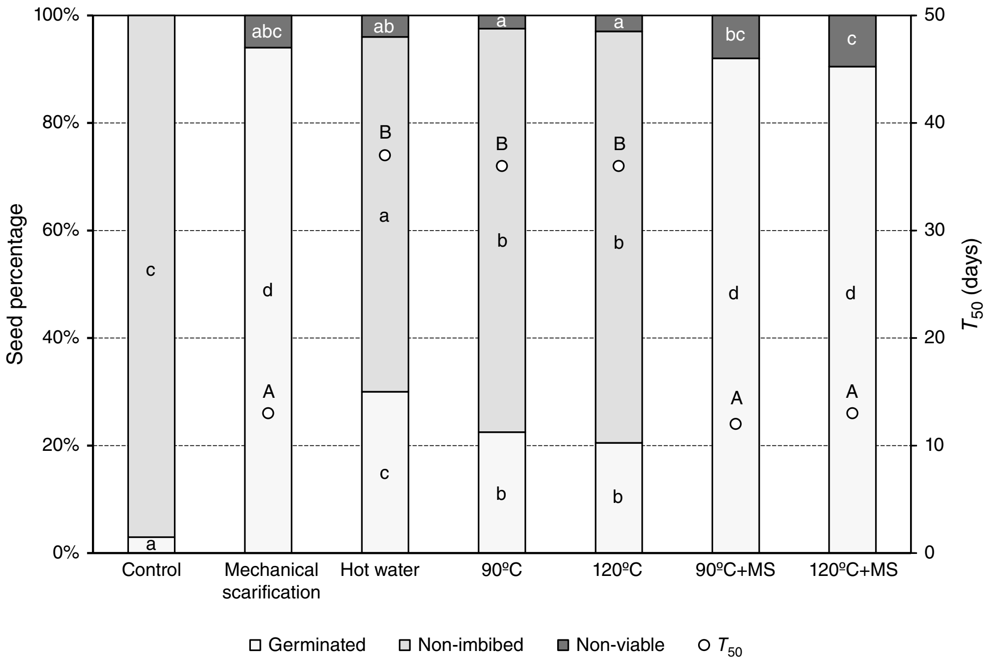

Fig. 1. Seed categories and germination speed (T_{50}, only if germination >10%) for *Adenocarpus argyrophyllus* seeds incubated at 20/7°C with photoperiod for 60 days after exposure to different treatments: control (no treatment), mechanical scarification (MS), hot water, dry heat (90°C) for 10 min, and dry heat (120°C) for 5 min. Non-germinated seeds were classified as non-imbibed and non-viable. Different lowercase letters represent significant differences ($P < 0.05$) between treatments per seed category. Different capital letters between columns denote significant differences in the T_{50} parameter.

scarification of the seed coat yielded similar results to those obtained with only mechanical scarification (Fig. 1).

Cumulative seedling emergence after heat shock of freshly matured seeds: intrapopulation variation

After monitoring for 5 years, the percentages of autumn and spring emergence were not significantly different under any treatment (Fig. 2).

Four months after sowing (December 2017), some seedlings emerged from seeds subjected to dry heat scarification (maximum of 7%) but not from the control seeds (Fig. 2). These results indicate the existence of a delay between the emergence of radicles and seedlings because after these thermal treatments, more than 20% of the radicles emerged after incubation for 2 months (Fig. 1).

On 1 June 2018, after the autumn and spring following sowing, seedling emergence increased considerably in all of the evaluated seed cohorts, where the values exceeded 20% in seed lots exposed to dry heat scarification before sowing (Fig. 2). The highest seedling emergence recorded in this study occurred during the following 2 years. The greatest relative increase occurred for the control seeds, but it was still significantly lower than the value corresponding to dry heat scarification treatment at 90°C/10 min. At the end of the evaluation period (December 2022; 5 years after sowing), the highest percentage emergence was achieved with the 90°C/10 min treatment (60.0 ± 3.5%), which was not significantly different compared with the control treatment (51.8 ± 4.3%) but significantly higher than that under 120°C/5 min (47.8 ± 5.7%) ($F_{2,87} = 5.42$; $P = 0.006$) (Table 1). Thus, there were no large differences between the final emergence percentages for each treatment, but seedling emergence during the first years was accelerated after scarification by dry heat (Fig. 2).

Five years after sowing, a considerable percentage of seeds did not emerge but a significant percentage still retained their viability. The mean percentages (different letters indicate significant differences between treatments) of seeds that remained viable at the end of the study were 21.8 ± 2.9%^{a,b} for control seeds, 17.5 ± 2.3%^{a} for those exposed to 90°C/10 min and 23.4 ± 3.2%^{b} for those exposed to 120°C/5 min ($F_{2,87} = 3.4$, $P = 0.04$) (Table 1).

Finally, high intrapopulation variation was found in the cumulative seedling emergence percentages among the different seed cohorts under each treatment. This variation was widest under the 120°C/5 min treatment, where the cumulative emergence percentage ranged between 26.3 (Plant 1) and 82.7% (Plant 7) (Fig. 2).

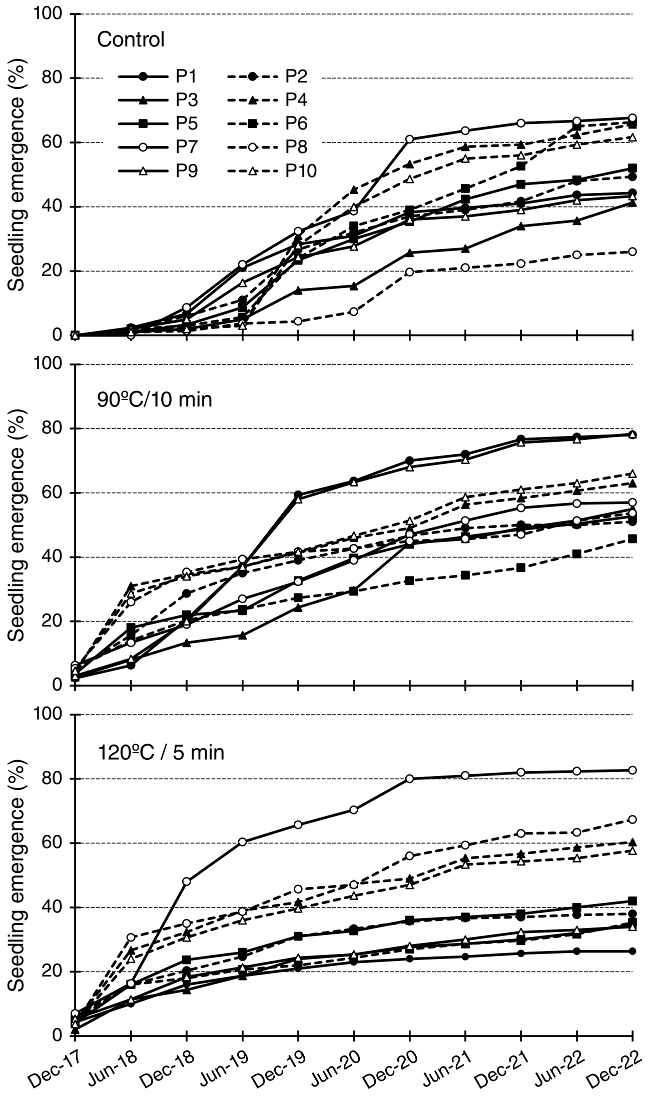

Fig. 2. Cumulative emergence over 5 years for *Adenocarpus argyrophyllus* seedlings from seeds subjected to three treatments: control, exposure to dry heat at 90°C for 10 min, and exposure to dry heat at 120°C for 5 min. Results were grouped into two dates in the year comprising December 1 and June 1 to reflect autumn and spring emergence, respectively. Seed cohorts from 10 plants are presented (from P1 to P10).

Cumulative seedling emergence after thermal shock of seeds recovered from soil

Dry heat scarification of seeds that had been buried in the soil for 2 years resulted in most of them producing seedlings at the end of spring following sowing. In particular, the cumulative seedling emergence percentage was 83% in seeds exposed to 90°C/10 min. For the control treatment, the final seedling emergence percentage was only 16% (Fig. 3).

Most of the seeds that did not produce seedlings at the end of these scarification treatments were non-viable (Table 2).

Soil seed bank

The soil seed bank estimated to a depth of 10 cm was 1770 ± 295 seeds/m^2 (Fig. 4). The percentage of viable seeds was very similar in the two soil layers that we analysed. The values were 547 ± 105 viable seeds/m^2 in stratum I (0–5 cm) and 415 ± 86 viable seeds/m^2 in stratum II (5–10 cm), with no significant difference ($F_{1,39} = 0.94$, $P = 0.34$).

Considering the two strata as a whole, the amounts of viable seeds per 1 m^2 obtained from the 20 sampled plots were highly dispersed, ranging from a single plot with no *A. argyrophyllus* seeds to three plots with densities greater than 2000 viable seeds/m^2.

Discussion

Our results showed that *Adenocarpus argyrophyllus* has the capacity to form large persistent soil seed banks. The following additional factors support the persistent character of *A. argyrophyllus* seeds: (1) the remarkable density of seeds estimated even in the deepest layer that we studied despite the shape and size of these seeds possibly hindering seed burial (Pérez-Fernández *et al.* 2002). This vertical soil distribution pattern for seeds is characteristic of long-term persistent seed banks (Thompson *et al.* 1997; Pausas and Lamont 2022). (2) Untreated seeds (control) continued to emerge 5 years after sowing and a significant proportion still remained viable in the soil. (3) The fraction (46%) of non-viable seeds detected in the soil seed population in the natural habitat compared with that recorded in pots after seed burial for 5 years (26%) allows us to infer a probable age of ~6–10 years for the natural soil seed bank, and thus it can be classified as a long-term persistent seed bank (Bakker *et al.* 1996; Thompson *et al.* 1997).

Germination with scarification treatments

The germination capacity determined for seeds not subjected to scarification in the present study was similar to that recorded in other legumes (Jastrzębowski *et al.* 2017; Mira *et al.* 2017). Only a small fraction (3%) of seeds germinated without the need for any treatment (control seeds), but these seeds are of great importance because they may never become dormant (Moreno Marcos *et al.* 1992). This fraction of soft-coated seeds comprises an efficient source for seedling establishment that contributes to maintaining population levels of this species without awaiting delayed seed coat deterioration or disturbance events such as fire. Species can colonise a high number of niches, horizontally, vertically and temporarily by producing both dormant and non-dormant seeds (Pérez-Fernández *et al.* 2002).

By contrast, the proportion of hard-coated seeds that required scarification was much higher. Many natural

Table 1. Intrapopulation variability in seedling emergence and seed viability in *Adenocarpus argyrophyllus*.

Plant no.	Treatment	Cumulative emergence (%)	Categories of non-emerged seeds (%)			Viability of non-emerged seeds (%)	
			Non-imbibed (viability)	Imbibed (viability)	Not recovered	Viable	Non-viable
1	Control	44.3 ± 4.5^{b,c,d}	27.3 ± 1.2 (20.0 ± 1.0)	16.0 ± 3.1 (5.3 ± 0.3)	12.4 ± 0.9	25.3 ± 0.7	30.4 ± 4.7^{b,c}
	90°C/10 min	78.0 ± 1.2^{e}	8.7 ± 0.7 (6.3 ± 1.2)	4.3 ± 1.2 (2.0 ± 1.0)	9.0 ± 0.6	8.3 ± 0.9	13.7 ± 1.7^{a}
	120°C/5 min	26.3 ± 3.0^{a}	50.3 ± 4.4 (29.3 ± 1.8)	7.0 ± 1.0 (3.0 ± 0.6)	16.0 ± 1.2	32.4 ± 2.0	41.3 ± 1.2^{e}
2	Control	49.3 ± 4.3^{c,d}	30.3 ± 5.8 (13.7 ± 2.7)	8.3 ± 0.9 (3.7 ± 0.7)	12.0 ± 1.2	17.3 ± 2.6	33.3 ± 3.7^{c}
	90°C/10 min	51.0 ± 4.4^{a,b}	25.7 ± 2.7 (14.7 ± 2.6)	9.7 ± 0.9 (3.7 ± 1.2)	13.7 ± 0.9	18.3 ± 3.8	30.7 ± 3.5^{b}
	120°C/5 min	38.0 ± 2.5^{b}	36.7 ± 2.0 (20.3 ± 3.2)	9.3 ± 2.4 (3.0 ± 0.6)	16.0 ± 1.2	23.3 ± 3.3	38.7 ± 4.8^{d,e}
3	Control	41.3 ± 3.4^{b}	34.7 ± 4.1 (25.7 ± 3.3)	10.7 ± 0.9 (6.0 ± 1.2)	13.3 ± 0.9	31.7 ± 2.2	27.0 ± 2.3^{a,b,c}
	90°C/10 min	55.0 ± 4.5^{b,c}	27.3 ± 2.0 (19.3 ± 1.5)	8.0 ± 1.2 (3.3 ± 0.3)	9.7 ± 1.9	22.7 ± 1.5	22.3 ± 5.7^{a,b}
	120°C/5 min	34.7 ± 2.3^{a,b}	39.0 ± 2.0 (26.0 ± 2.1)	12.0 ± 1.2 (5.0 ± 0.6)	14.3 ± 1.2	31.0 ± 2.3	34.3 ± 1.2^{c,d,e}
4	Control	65.7 ± 2.0^{e}	16.7 ± 1.8 (12.0 ± 1.5)	7.7 ± 0.3 (3.3 ± 0.3)	10.0 ± 0.6	15.3 ± 1.9	19.0 ± 0.6^{a,b}
	90°C/10 min	63.0 ± 1.5^{c,d}	20.0 ± 1.2 (11.0 ± 0.6)	8.7 ± 0.7 (4.3 ± 0.3)	8.3 ± 0.9	15.3 ± 0.9	21.7 ± 0.9^{a,b}
	120°C/5 min	60.3 ± 3.5^{c,d}	20.7 ± 2.4 (11.7 ± 0.9)	8.7 ± 0.7 (4.0 ± 0.6)	10.3 ± 0.9	15.7 ± 1.5	24.0 ± 3.0^{a,b,c}
5	Control	52.0 ± 3.0^{d}	25.7 ± 2.3 (18.7 ± 1.5)	10.3 ± 0.9 (4.3 ± 0.3)	12.0 ± 0.6	23.0 ± 1.7	25.0 ± 1.7^{a,b,c}
	90°C/10 min	52.7 ± 1.8^{a,b}	24.7 ± 3.3 (17.0 ± 3.1)	10.3 ± 1.2 (4.3 ± 0.7)	12.3 ± 0.9	21.3 ± 2.7	26.0 ± 1.2^{a,b}
	120°C/5 min	42.0 ± 5.7^{b}	35.3 ± 4.4 (26.7 ± 2.8)	9.0 ± 0.6 (4.3 ± 0.3)	13.7 ± 1.5	31.0 ± 3.0	27.0 ± 2.9^{b,c,d}
6	Control	66.3 ± 0.7^{e}	16.7 ± 0.3 (9.7 ± 0.9)	8.0 ± 0.6 (3.3 ± 0.3)	9.0 ± 0.6	13.0 ± 1.2	20.0 ± 0.7^{a,b}
	90°C/10 min	45.7 ± 2.9^{a}	34.0 ± 2.5 (25.3 ± 2.2)	8.0 ± 0.6 (4.0 ± 0.7)	12.3 ± 0.9	29.3 ± 2.3	25.0 ± 0.6^{a,b}
	120°C/5 min	35.3 ± 1.9^{b}	39.0 ± 1.5 (28.3 ± 0.9)	12.3 ± 0.9 (5.3 ± 0.3)	13.3 ± 0.9	33.7 ± 1.2	31.0 ± 1.7^{c,d,e}
7	Control	67.7 ± 0.9^{e}	17.3 ± 0.3 (11.0 ± 0.6)	7.0 ± 0.6 (3.3 ± 0.3)	8.0 ± 0.6	14.3 ± 0.3	18.0 ± 1.0^{a}
	90°C/10 min	57.0 ± 1.5^{b,c}	26.0 ± 2.1 (18.0 ± 1.5)	6.0 ± 0.6 (2.3 ± 0.3)	11.0 ± 0.6	20.3 ± 1.5	22.7 ± 0.3^{a,b}
	120°C/5 min	82.7 ± 1.8^{e}	6.3 ± 2.2 (3.3 ± 1.2)	4.0 ± 0.6 (2.0 ± 0.6)	7.0 ± 0.6	5.3 ± 0.7	12.0 ± 1.2^{a}
8	Control	26.0 ± 1.7^{a}	48.3 ± 1.9 (38.0 ± 1.2)	11.0 ± 0.6 (3.7 ± 0.3)	14.7 ± 1.8	41.7 ± 0.9	32.3 ± 1.7^{c}
	90°C/10 min	53.7 ± 1.5^{a,b}	22.3 ± 1.9 (15.0 ± 2.1)	14.0 ± 1.2 (6.0 ± 0.6)	10.0 ± 0.6	21.0 ± 1.5	25.3 ± 1.8^{a,b}
	120°C/5 min	67.3 ± 1.8^{d}	18.3 ± 1.8 (10.7 ± 0.9)	7.3 ± 0.3 (3.7 ± 0.3)	7.0 ± 0.6	14.3 ± 1.2	18.3 ± 0.9^{a,b}
9	Control	43.3 ± 1.2^{b,c}	32.0 ± 1.2 (1.9 ± 0.6)	12.0 ± 1.2 (4.0 ± 0.6)	12.7 ± 0.9	23.0 ± 0.0	33.7 ± 1.2^{c}
	90°C/10 min	78.3 ± 1.3^{e}	7.7 ± 1.3 (3.7 ± 0.9)	5.0 ± 0.6 (1.7 ± 0.3)	9.0 ± 0.6	5.3 ± 1.2	16.3 ± 0.3^{a}
	120°C/5 min	34.0 ± 1.7^{a,b}	43.0 ± 3.2 (30.0 ± 1.5)	9.3 ± 1.5 (2.7 ± 0.3)	13.7 ± 0.9	32.7 ± 1.3	33.3 ± 0.9^{c,d,e}
10	Control	61.7 ± 1.2^{e}	19.0 ± 0.6 (10.0 ± 0.6)	8.3 ± 0.9 (3.3 ± 0.3)	11.0 ± 0.6	13.3 ± 0.3	25.0 ± 1.2^{a,b,c}
	90°C/10 min	66.0 ± 5.3^{d}	17.3 ± 3.2 (9.7 ± 1.9)	8.3 ± 1.5 (3.7 ± 0.3)	8.3 ± 0.9	13.3 ± 1.8	20.7 ± 3.5^{a,b}
	120°C/5 min	57.7 ± 2.3^{c}	21.7 ± 3.0 (11.3 ± 0.9)	10.0 ± 1.2 (3.7 ± 0.3)	10.7 ± 0.9	15.0 ± 0.6	30.7 ± 1.9^{c,d,e}

Seed cohorts from 10 plants were analysed. The table shows mean seedling emergence percentages (±s.e.) and categories of non-emerged seeds (viability in brackets) recorded 5 years after sowing. Freshly matured seeds were previously scarified using two dry heat treatments and compared with unheated (control) seeds. In the columns for cumulative emergence and non-viable seeds under each treatment, different letters indicate significant differences between the means corresponding to different plants ($P < 0.05$).

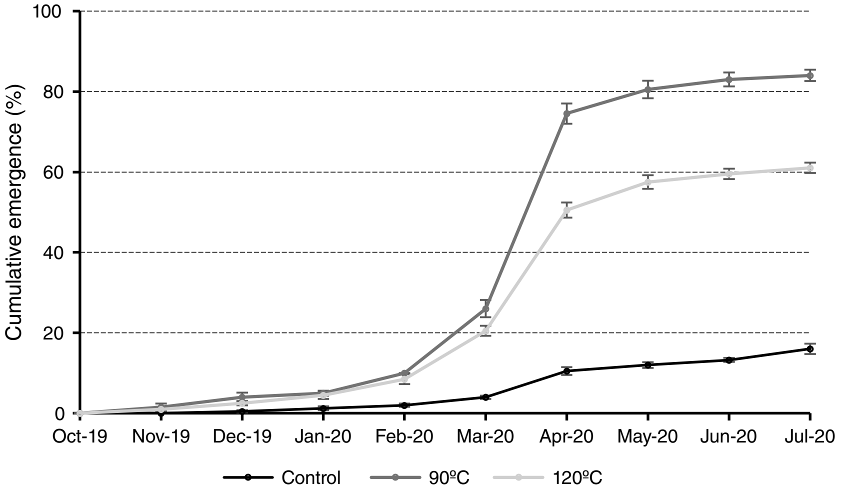

Fig. 3. Cumulative emergence (mean ± s.e.) of *Adenocarpus argyrophyllus* seedlings after dry heat scarification (90°C/10 min and 120°C/5 min) of seeds recovered from the soil 2 years after sowing.

Table 2. Seedling emergence and viability of *Adenocarpus argyrophyllus* seeds recovered from soil.

Treatment	Cumulative emergence (%)	Categories of non-emerged seeds (%)			Viability of non-emerged seeds (%)	
		Non-imbibed (viability)	Imbibed (viability)	Not recovered	Viable	Non-viable
Control	16.0 ± 1.3^a	76.9 ± 1.6 (73.7 ± 1.1)	2.3 ± 0.6 (1.5 ± 0.5)	4.8 ± 0.5	75.2 ± 2.1	8.8 ± 1.2^a
90°C/10 min	84.0 ± 1.4^c	9.0 ± 0.6 (5.5 ± 1.0)	2.5 ± 0.5 (1.0 ± 0.6)	4.5 ± 0.5	6.5 ± 1.3	9.5 ± 1.0^a
120°C/5 min	61.0 ± 1.3^b	16.0 ± 0.8 (8.0 ± 0.8)	10.0 ± 0.8 (2.0 ± 0.8)	13.0 ± 1.3	10.0 ± 1.4	29.0 ± 1.3^b

Seeds recovered from the soil were previously scarified using two dry heat treatments and compared with unheated (control) seeds. The table shows mean seedling emergence percentages (±s.e.) and those for categories of non-emerged seeds (viability in brackets). In the columns for cumulative emergence and non-viable seeds, different letters indicate significant differences between means ($P < 0.001$).

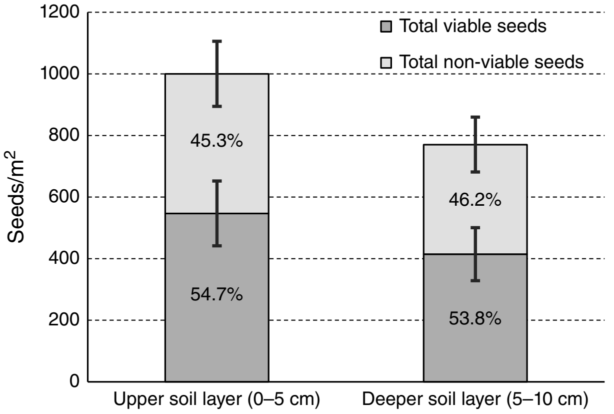

Fig. 4. Soil seed bank density (mean ± s.e.) for *Adenocarpus argyrophyllus*. Two depth strata were differentiated and the percentages of viable and non-viable seeds are indicated for each.

mechanisms can crack the tegumentary barrier in legumes, such as temperature oscillation, wet–dry cycles (Rolston 1978; Ferrandis *et al.* 1999*a*), soil microbial activity and herbivore digestion (Pereiras *et al.* 1985; Robles *et al.* 2005). The heat generated by wildfires is also an important trigger factor (Baskin and Baskin 2014; Pausas and Lamont 2022), although according to very recent studies, it may not be the most significant (Rosbakh *et al.* 2023). Heat fractures special heat-sensitive tissues in the seed coat (Gama-Arachchige *et al.* 2013; Brits and Manning 2019) to allow the uptake of water, which explains the massive germination of legumes recorded from hard-coated seeds stored in persistent soil banks after some wildfires, as found with *Erophaca baetica* subsp. *baetica* (L.) Boiss. (Clemente *et al.* 1996), *Bituminaria bituminosa* (L.) C.H. Stirt., *Argyrolobium zanonii* (Turra) P.W. Ball, *Dorycnium pentaphyllum* Scop. (Herranz *et al.* 1998), *Cytisus scoparius* and *Retama sphaerocarpa* (L.) Boiss. (Magaña Ugarte *et al.* 2021). However, these same high temperatures can kill the seeds of some species (Ocampo-Zuleta *et al.* 2022).

Promotion of delayed release from dormancy over time by thermal shock

In the present study, the stimulatory effect of thermal treatments on germination in the short term (22% after 2 months of incubation) was similar to that obtained in another species in the genus comprising *Adenocarpus lainzii* (Castrov.) Castrov. (Rivas *et al.* 2006), but lower than that obtained in other legumes such as *Ulex europaeus* L. (Pereiras *et al.* 1985), *Genista florida* and *Cytisus scoparius* (Tarrega *et al.* 1992), *Cytisus striatus* (Hill) Rothm. and

C. reverchonii (Degen and Hervier) Bean (Herranz *et al.* 1998), and *Genista berberidea* Lange and *G. triacanthos* Brot. (Rivas *et al.* 2006). However, our results suggest that these and other previous studies based on similar designs may have interpreted their findings in a biased manner because evaluating treatments only in the immediate term after they were applied might not have been sufficient to observe the total effects and to assess the complete population consequences of the thermal treatments. By contrast, in the present study, we found that compared with the control seeds, seedling emergence was stimulated in freshly matured seeds after exposure to heat shock and remaining buried in the soil for a substantial time (≥10 months). The cumulative seedling emergence percentage after 5 years was very similar under all treatments and higher than 50%, but the increased seedling emergence after heat shock during the first years could facilitate colonisation by *A. argyrophyllus* plants and improve their competitive capacity relative to other species. These results demonstrate the importance of evaluating the effects of fire on soil seed banks in the medium to long term after fires (Shi *et al.* 2022).

Our findings suggest that seeds could undergo weakening of any coat region during thermal treatment, which would facilitate their fracture or degradation by any of the environmental factors mentioned above (Brits and Manning 2019). The post-fire depletion of the short-term seed bank of *Cistus salviifolius* recorded in the 2–5 cm deep soil layer without stimulation by heat was explained in a similar manner by Ferrandis *et al.* (1999*a*). It was shown that wet heat (incubation under wet, warm to hot conditions between 25 and 40°C) was an important dormancy-release mechanism for seeds of the leguminous tree *Parkinsonia aculeata* buried at a depth of 2 cm, although release occurred slowly (Van Klinken *et al.* 2008). In the shade house used in the present study, the average maximum temperatures during July and August ranged between 32 and 33°C, so wet heat could have been of great importance for definitive dormancy release in seeds weakened by thermal treatment and it may have been responsible for the emergence of seedlings recorded in the autumn.

Natural seedling recruitment is mainly observed in the autumn for *A. argyrophyllus* (Benito 2013), but our results showed that emergence of seedlings occurred in both the autumn and spring, which could be a seedling recruitment bet-hedging strategy in order to adapt to the continentality of the climate, with stressful conditions due to both cold and drought, rather than to adapt to the fire regime (Pausas *et al.* 2022).

Intrapopulation variation

The responses of the different seed cohorts to dry heat scarification exhibited great intrapopulation variation, as previously observed in *Ceratonia siliqua* L. (Pérez-García 2009) and some Australian shrub legumes (Liyanage and Ooi 2015). The specific thermal treatment that promoted the highest seedling emergence (even in the control) varied among individuals, which may reflect high genetic variability that could provide an advantage under conditions with frequent disturbances and large fluctuations in environmental factors (Moreno Marcos *et al.* 1992). During wildfires, the heat conditions in soil are not uniform in space and time (Odion and Davis 2000; Pausas and Lamont 2022), and thus the range of temperature conditions commonly encountered in the soil surface horizons during fires can vary greatly at different points (Tarrega *et al.* 1992; Odion and Davis 2000; Huerta *et al.* 2022). When a fire occurs, the presence of seeds from different plants with different responses to a thermal stimulus means that a fairly high number of hard-coated seeds are always released from physical dormancy irrespective of the intensity and duration of the fire (Thanos and Georghiou 1988). Other fine-scale factors that promote seed-coat permeabilisation (e.g. opening of the understorey resulting in direct insolation of the soil) may be even more spatiotemporally heterogeneous than the fire's intensity, so variability in individual responses may also be advantageous in this context. Thus, intrapopulation variation combined with the explosive dispersal of seeds increasing the spatial heterogeneity of their distribution in the soil may have great adaptive value (Liyanage and Ooi 2015). The maximum explosive dispersion distance in Leguminosae is positively correlated with plant height, and the reported distances range from 4 m in *Cytisus multiflorus* (L'Hér.) Sweet (Moreno Marcos *et al.* 1992), which is smaller than *A. argyrophyllus*, to tens of metres in the tree legume *Tetraberlinia moreliana* Aubrev. (Van Der Burgt 1997).

Sensitivity of seeds from the soil bank to thermal shocks

The emergence rate increased for seeds recovered from the soil 2 years after burial and sown again following a thermal shock compared with the freshly matured seeds. During the period of previous burial, it is likely that the aforementioned environmental factors weakened the seed coat and increased the effectiveness of the thermal shock treatment. Germination stimulation similar to that obtained in the present study could be produced by high temperatures during wildfires affecting seeds stored in the soil as a persistent seed bank, and this may explain the very high levels of germination recorded for legumes after some wildfires (Pausas *et al.* 2022).

The germination rates after thermal pretreatment were much slower than those for mechanically scarified seeds. This pattern has been recorded previously in Leguminosae species (Tarrega *et al.* 1992; Herranz *et al.* 1998) and Cistaceae species (Thanos *et al.* 1992; Herranz *et al.* 1999). These differences can be explained by the different effects of mechanical scarification and thermal pretreatment on the seed coat structures in species with hard-seededness

(Thanos *et al.* 1992). The slow germination rate of seeds softened by heat is considered an obvious ecological advantage under dry summer and fire-prone Mediterranean climatic conditions (Thanos and Georghiou 1988; Thanos *et al.* 1992), as it can prevent post-fire germination after occasional summer rains.

Conclusions

Persistent soil seed banks are important for ensuring population persistence, particularly in ecosystems that are subjected to variable or temporary stochastic disturbance regimes with harsh environmental conditions (Thompson *et al.* 1997; Fenner and Thompson 2005), as found in much of the Mediterranean region, with pronounced continentality, periods of drought and frequent wildfires (Ferrandis *et al.* 1999*b*; Martínez-Duro *et al.* 2010). In the present study, we demonstrated the importance of persistent soil seed banks from another viewpoint because their presence could give seeds sufficient time to exhibit the actual stimulatory effects of fire or other disturbances such as removing plant cover in the Mediterranean context. This delayed effect may constitute a bet-hedging strategy by spreading the probability of recruitment over time (Ooi *et al.* 2009; Ooi 2012), so the chances of seedling survival is increased by not all seeds germinating simultaneously after a disturbance, thereby avoiding less competition at the critical moment of germination. Moreover, we showed that seeds stored in the soil for 2 years responded more rapidly to thermal stimuli caused by wildfires than freshly matured seeds. Therefore, it would be interesting to conduct more detailed studies of the influence of the storage time in soil on dry heat scarification effects in seeds with physical dormancy.

In addition to soil seed banks, past exposure to fire in fireprone regions has selected for other adaptations that allow plants to persist, including hard-seededness, which provides protection for the embryo, and that facilitate heat-induced germination, which favours the formation of persistent soil seed banks (Thanos *et al.* 1992), as we showed for *A. argyrophyllus*. Another of these adaptations may be intrapopulation variability in terms of the germination response to exposure to dry heat, as demonstrated in the present study, which could ensure the emergence of post-fire seedlings throughout an affected area regardless of the intensity of the fire.

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Author affiliations

[A]Department of Plant Production and Agricultural Technology, University of Castilla-La Mancha, Higher Technical School of Agronomic and Forestry Engineering and Biotechnology, University Campus s/n, Albacete 02071, Spain.

[B]Botanic Institute, University of Castilla-La Mancha, Botanic Garden of Castilla-La Mancha, Avenida de la Mancha s/n, Albacete 02006, Spain.