Plant Ecol (2017) 218:1313–1324
DOI 10.1007/s11258-017-0770-6

What we don't seed: the role of long-lived seed banks as hidden legacies of invasive plants

Isabel Passos · Hélia Marchante · Rosa Pinho · Elizabete Marchante

Received: 16 January 2017 / Accepted: 25 September 2017 / Published online: 5 October 2017

Abstract *Acacia dealbata* is an invasive south-eastern Australian tree that produces a persistent soil seed bank. In order to characterize the seed bank in plots invaded by *A. dealbata* and to understand its implications for management and re-invasion risk, density, germinability, and viability of the seed bank were evaluated in five sites in central Portugal. Soil samples were collected in *A. dealbata* invaded plots and adjacent areas and screened for seeds, which were then quantified and germinated at 25 °C. A subset of seeds was first exposed to 60 °C to assess the effect of high soil temperature on dormancy breaking. Variables influencing differences between sites were explored with generalized linear mixed models with a Poisson distribution. Inside *A. dealbata* invaded plots the seed bank averaged 4608 seeds/m^2, reaching up to 62,747 seeds/m^2; in adjacent areas, up to 14 m from the plots, only 9 seeds/m^2 were found. Seed bank densities were mostly influenced by stoniness, number of fires in the last 10 years, and density of trees and roots. Almost 90% of seeds were viable, but only 8.6% germinated without treatment. Nearly 70% of seeds exposed to 60 °C germinated without any physical stimulation, suggesting that high soil temperatures can effectively break seed dormancy. The high density and viability of *A. dealbata*'s seed bank and its ability to disperse seeds far from the parent plants contribute to the species' invasive success. These features combined with heat events that can overcome seed dormancy need to be considered in the management of this species.

Keywords Seed bank · Seed germinability · Seed viability · Invasive plants management · Seed dormancy · Acacia dealbata

Introduction

Invasive species are considered as one of the major threats to biodiversity (Richardson et al. 2008; Vilà et al. 2011) with additional negative impacts on human health, society, and economy worldwide (Andreu and Vilà 2007). In the case of alien plants, prolific seed production is one of the key features that significantly

---

Communicated by Thomas Abeli.

I. Passos · R. Pinho
Department of Biology, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

H. Marchante
Department of Environment, Escola Superior Agrária de Coimbra, Instituto Politécnico de Coimbra, 3040-316 Coimbra, Portugal

H. Marchante · E. Marchante (✉)
Department of Life Sciences, Centre for Functional Ecology, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
e-mail: emarchante@uc.pt

contributes to their successful invasion outside their native range (Richardson and Kluge 2008; Gibson et al. 2011). If, in addition, seeds are long-lived this can lead to the accumulation of extensive soil seed banks that may remain as a “hidden legacy” for a long time and eventually jeopardize management efforts (Richardson and Kluge 2008; Le Maitre et al. 2011; Gibson et al. 2011; Gioria et al. 2012; Wijayabandara et al. 2013).

In spite of considerable and increasing efforts to manage invasive plants, management programs often remove the existing stands (Le Maitre et al. 2011) without considering the soil seed bank. However, when dealing with species with large and long-lived seed banks, this approach often fails to achieve successful control (D’Antonio and Meyerson 2002; Le Maitre et al. 2011; Marchante et al. 2011a) because massive quantities of seeds germinate afterwards (Marais et al. 2004; McConnachie et al. 2012). With such species, control efforts can only be successful if a significant reduction of the seed bank is achieved (Marchante et al. 2011a; Gioria et al. 2012).

One of the most aggressive invasive plants in Portugal is *Acacia dealbata* Link (silver wattle, *Fabaceae*), which represents a major conservation problem (Fernandes 2008; Correia et al. 2014; Marchante et al. 2014). This fast growing south-eastern Australian tree can grow up to 30 m and was introduced in different parts of the world, predominantly for forestry and as an ornamental tree, becoming invasive in some parts of Europe (Lorenzo et al., 2010; Marchante et al. 2014), South America (Fuentes-Ramirez et al. 2011), North America, southern Africa, Indian Ocean islands, New Zealand, and even Western Australia (Richardson and Rejmánek 2011). In its native range, *A. dealbata* occurs in forests, woodlands, grasslands, and riparian ecosystems on a wide variety of soils (Maslin and McDonald 2004; DAISIE 2006). In its invaded range, it is capable of occupying a vast altitudinal range and a great variety of soil types and habitats, from sand dunes to forests and urban areas (Lorenzo et al. 2010; Marchante et al. 2014). Like most *Acacia* species, *A. dealbata* produces a large number of seeds and a larger seed crop every 2–3 years, starting to produce seeds within 4 or 5 years (Maslin and McDonald 2004; Richardson and Kluge 2008). The seeds are probably dispersed into the soil by ants, *i.e.*, myrmecochory (Richardson and Kluge 2008). Published data about the seed bank of *A. dealbata* are scarce (Lorenzo et al. 2010; Gioria et al. 2012), but Gibson and colleagues (2011) refer to seed rain of 2553 seeds/m^2/year, and a stored seed bank ranging from 10,000 to 22,500 seeds/m^2. Almost 80% of these seeds are stored within the first 10 cm of soil and can remain viable for over 50 years due to a water impermeable coat, which confers them the ability to remain dormant (Richardson and Kluge 2008). The germination of *A. dealbata* seeds is most often triggered by heat (Adair 2008a), which is known to break dormancy of *Acacia* seeds (Richardson and Kluge 2008; Wilson et al. 2011). Additionally, germination may be promoted by positive plant–soil feedbacks resulting from changes in soil conditions (Rodríguez-Echeverría et al. 2013). These features give the species a very high potential for regeneration, especially in fire prone habitats.

Most wildfires can reach very high temperatures, and during these events, soil can reach temperatures from about 60 °C to over 800 °C (Stoof et al. 2013). In the daytime in summer and during heat waves, high soil temperatures (40–60 °C) can also be reached and have already been documented to break seed dormancy of some species (Ooi et al. 2012, 2014; Santana et al. 2010, 2013). Some authors suggest that 60 °C is enough to break the seed coat of *Acacia* species (Bradstock and Auld 1995) while others state that such a temperature is not high enough (Saharjo and Watanabe 1997; Ooi et al. 2014). Germination of *Acacia* seeds is also associated with other disturbances, such as vegetation clearing, construction activities, roads, vehicle traffic, and trampling (D’Antonio and Meyerson 2002; Gibson et al. 2011; Le Maitre et al. 2011). *Acacia dealbata* has also been identified in sites with no signs of recent fires or other kinds of disturbances (Fuentes-Ramirez et al. 2011; Lorenzo et al. 2012), suggesting the existence of additional stimuli that may work as facilitators of seed germination.

Control of *A. dealbata* is usually done by removing the existing plants, by either mechanical or chemical methods. However, even when the most effective control methods are applied, successful control is seldom achieved because follow-up treatments are often insufficient to deal with regeneration from seeds or resprouting and the accumulated soil seed bank is ignored (Fernandes, 2008; Sanz-Elorza et al. 2004; *authors personal observation*).

In order to support the sustainable management of *A. dealbata* and considering its long-lived seed bank, it is essential to study the density of this seed bank and the factors that influence it. In this context, the main goal of our work was to quantify and characterize the seed bank in plots invaded by *A. dealbata*, and to understand its implications in terms of management and risk of re-invasion. Furthermore, as seeds are known to be dormant, we wanted to understand if high temperatures or heat waves (around 60 °C) were enough to stimulate seed germination, as this can trigger invasions without the need of further disturbances. As a secondary goal, we also explored if there were seeds outside the plots of *A. dealbata*, since the existence of seeds in these areas can promote new invasions thereby increasing the invaded area. Because *A. dealbata* produces many long-lived seeds that can be dispersed by ants, we expect to find a numerous and viable seed bank under and close to the invaded plots and at least some seeds outside these areas. Furthermore, since new invasions by this species occur in non-disturbed and non-burned areas, we expect high soil temperatures to be able to break dormancy and promote seed germination.

Methods

Study area

This study took place in central Portugal (Fig. 1) where *A. dealbata* has been present for a long time and where it has been spreading rapidly in recent years, dominating large areas. New invasions are frequent, some of them near important conservation areas, such as the Natural Park of Serra da Estrela or Serra da Gardunha, where important populations of rare plant species and endemic habitats are known (ICN 2006; Meireles et al. 2013).

The climate is Mediterranean with large daily temperature amplitude. The annual mean temperature varies between 11.2 °C (Weather station no. 082, Guarda, Lat.: 40°32′N; Lon.: 07°16′W, period 1981–2010) and 15.9 °C (Weather station no. 507, Castelo Branco, Lat.: 39°50′N; Lon.: 07°28′W, period 1981–2010). It can rise up to a maximum of 41.6 °C in the summer with a minimum of −10.8 °C in the winter. The annual average precipitation ranges from 780 to 920 mm. Soils are acid, dominated by granite and shale. Maximum soil temperature at 5 cm deep has been registered up to 49 °C during summer (Horta 2014).

Within the study area, five sites were selected (A, B, C, D, and E, Fig. 1), based on five criteria: (1) only one *Acacia* species (*A. dealbata*) was present to avoid confusion with seeds of other *Acacia* species that have similar seeds; (2) plots were well individualized so that the limits could be easily identified, assuring that there were no scattered *A. dealbata* individuals outside the plots that may work as sources of seeds; (3) plots were at least 300 m^2 in order to guarantee that the inner soil samples were central to the plot; if plots were smaller, the inner samples may have been located near the opposite margin of the plot; (4) vegetation surrounding the *A. dealbata* plot was scrubby vegetation, mostly dominated by *Cytisus* spp., and at least 20 m wide; and (5) accessibility to the site was easy considering the need to carry heavy soil samples. Soil samples were collected in plots of *A. dealbata* where cover reached between 80 and 100% (hereafter called "acacia plots") and in contiguous areas without *A. dealbata* outside these plots (hereafter termed "adjacent areas"). There were no records or evidence of planting of *A. dealbata* in any of the study sites and as such it was assumed that the species spread by its own means to these areas. Fire is a constant element throughout the study area and all sites had burned in the last 10 years, the most recent being a fire in 2008 in sites B and C (Table 1). Consequently, individuals present in these areas were not very old or big. Acacia plots with these characteristics are very prevalent in Portugal, due to the high frequency of fires. Several parameters were registered for each study site in order to explore the variables influencing seed bank density (Table 1).

Seed bank density

Soil sampling was conducted between February and April 2013, during flowering of *A. dealbata* and before seed rain. In each sampling site, three 30-m-long transects were set (Fig. 2), in different directions, at least 4 m apart from each other, but generally more. The point under the first *A. dealbata* branch was considered the limit of the acacia plot (0 m). To evaluate the seed bank inside acacia plots, 10 samples were collected under the trees, in each transect, at 1-m intervals (samples designated as "−"). In adjacent areas, 10 soil samples were collected, at 2-m intervals (samples designated as "+"), in order to access seed

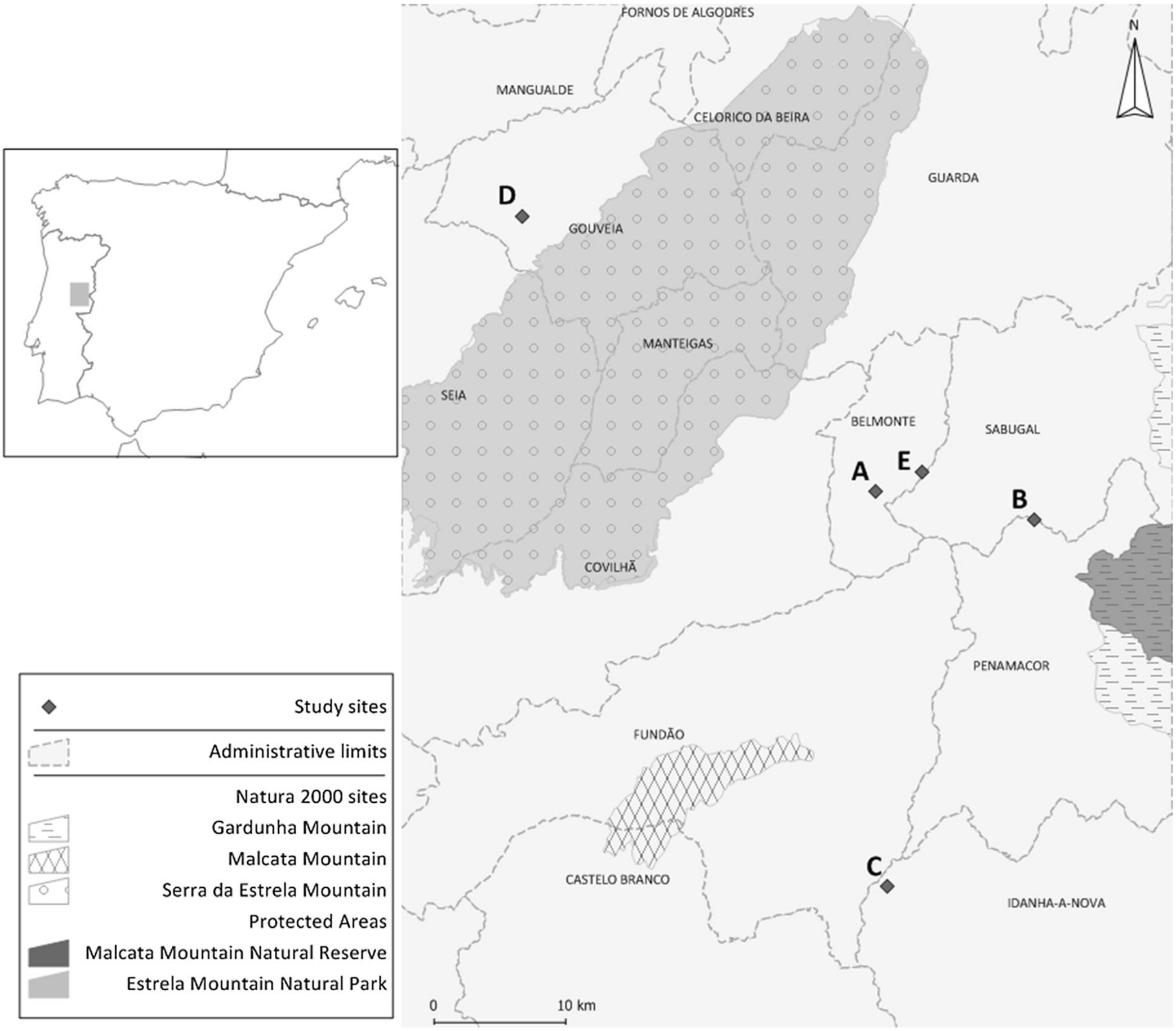


Fig. 1 Location of *Acacia dealbata* sampling sites (A, B, C, D, E) in central Portugal

dispersion distance (Fig. 2). Sampling in adjacent areas was not intensive, aiming only to detect the presence of seeds in these areas and not to fully characterize the *A. dealbata* seed bank outside the plots; considering the extent and heterogeneity of such areas a full characterization of the seed bank would require a much greater sampling effort. In each transect 20 samples were collected, totalling 60 samples per site (30 inside the acacia plot and 30 in the adjacent area). The first three samples collected inside the acacia plot were considered as the margin of the plot (samples −1, −2, −3) and the remaining were considered as the center (−4 to −10; Fig. 2).

For each sample, the top 10 cm of soil, including leaf litter, was collected using an 11-cm-diameter core. The samples were then dried and screened and the number of seeds counted; for that, soil was first passed through a 4-mm sieve, to remove rocks and plant remains, and the remaining soil was spread on white trays where the seeds were carefully searched for—*A. dealbata* seeds are black, have a regular shape, and are relatively large (ca. 4–5 mm long and 2–3 mm wide) and very easy to detect after some training; as such, we were confident that no seeds were missed.

Germinability and viability

Seeds extracted from soil samples were tested for germinability (for this, we considered seeds germinating without any physical stimulus) and viability

Table 1 Characterization of study sites, acacia plots, and soil samples

Variables		Site A	Site B	Site C	Site D	Site E
Location	Altitude (m)	490	580	340	430	510
	Exposure	E	S	E	NE	NE
	Slope (°)	18	24	18	6	10
	Soil	Granite	Shale	Granite	Granite	Granite
Acacia plots	Tree density (tree/m^2)	9	7	5	6	6
	Acacia dealbata coverage (%)	100	100	80	100	100
	Total vegetation coverage (%)	100	100	80	100	100
	Average trunk diameter at base (cm)	2.4	1.9	2.2	3.2	2.1
	Maximum trunk diameter at base (cm)	11.7	5.2	6.1	9.9	12.6
Soil samples characterization (mean)—see scale below[a]	*Acacia dealbata* pods	1.6	0.9	0.6	1.7	1.2
	Acacia dealbata leaves	1	1.2	0.8	1.2	1.2
	Root density	1.8	1.6	1.5	1.1	1.3
	Stoniness	0.8	1.3	1.4	1.1	1.3
Site disturbances	Last fire	2003	2008	2008	2003	2003
	Number of fires in the last 10 years	1	2	1	1	1
	Signs of previous cut[b]	1	0	0	0	1

Data were collected during sampling and used in the statistical analysis

[a] A qualitative scale was used to classify each sample and the mean value of the plot was then calculated (0—none; 1—few; 2—plenty)

[b] Only presence (1) or absence (0) was considered

(considering seeds germinating with and without physical stimulus) in 90-mm sterilized Petri dishes containing filter paper moistened with distilled water and kept in the dark. When more than 50 seeds were found per sample, a maximum of 50 seeds were tested (i.e., one Petri dish per sample). Germinability was assessed after seeds sterilization (Crisóstomo et al. 2007) at 25 °C with no further treatment, for 30 days. In addition, to check the influence of high soil temperatures in germination, a subset of the seeds from 36 samples, randomly selected, were submitted to an initial temperature of 60 °C for 10 h. After this initial heat treatment, seeds were incubated at 25 °C to allow normal germination. Seeds were checked out every 2 days, and germinated and rotted seeds were counted and eliminated. Seeds were considered germinated when the radicle was at least 2 mm long. After 30 days, non-germinated seeds were considered dormant and scarified (cut at the micropylar end with a scalpel) to test viability, and were observed for another 30 days. Seeds not germinated after this time were considered non-viable. Total viable seeds were the sum of all germinated seeds, without (first 30 days) or with physical stimulus (after the first 30 days).

Statistical analysis

An initial exploratory analysis of the density of seeds was performed to observe the distribution of seeds along transects. This analysis suggested differences between the center and the margin of the acacia plots and consequently the subsequent analyses were performed considering these two areas. Two distinct analyses were performed. Firstly, taking into account the number of seeds (density), germinability, and viability as dependent variables, we tested for differences between: (i) the number of seeds in the acacia plots and the adjacent areas, (ii) the number of seeds in the margin and the center of the acacia plots, and (iii) seeds germinating at different temperature treatments and with and without physical stimulus. For each response variable, an analysis of deviance (GLM) with Poisson distribution was used. Percentage data were arcsine transformed.

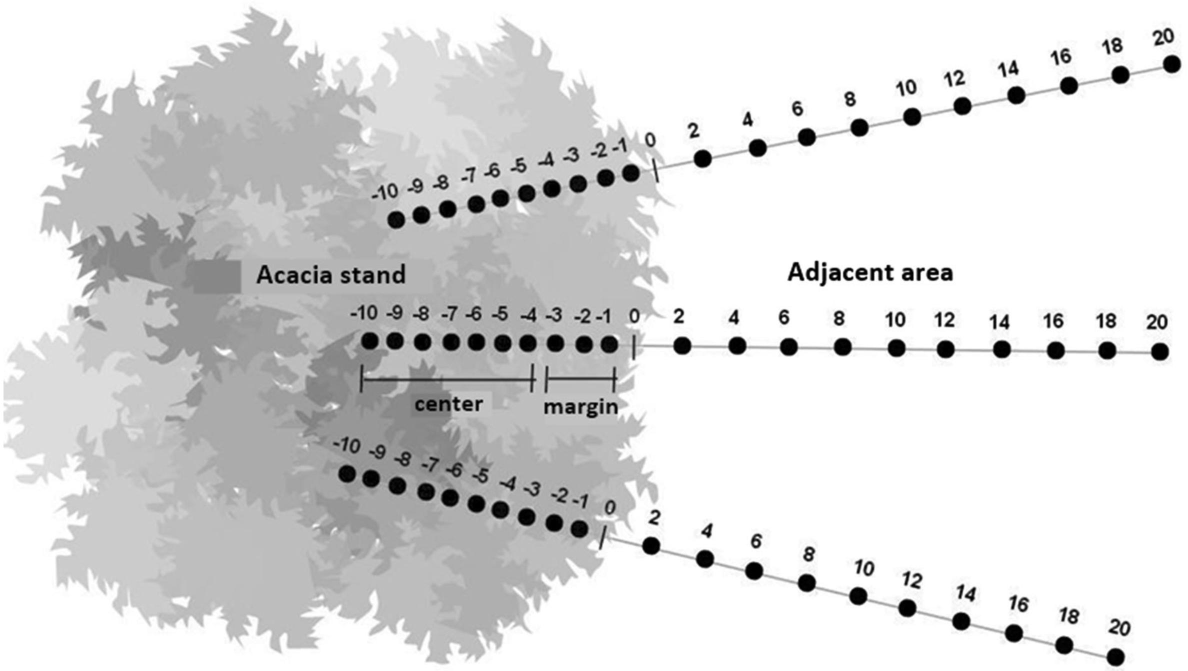


Fig. 2 Experimental set-up of transects established to evaluate the seed bank, with 10 m lying inside the acacia plot (0 to −10 m) and 20 m in the adjacent area (0–20 m); the point under the first *A. dealbata* branch was set as the limit of the plot (point zero) and the margin of the plot considered from −3 to 0 m. Samples were collected with 1-m interval inside the acacia plot and 2-m interval in the adjacent area. Transects were established in different directions and the minimum distance between transects was 4 m

To explain differences in seed density between study sites, we fitted a generalized linear mixed model (GLMM) with a Poisson distribution. Given that the response data were non-independent, we fitted all models using "transect" as a random factor (Bolker et al, 2008). A set of 17 explanatory variables were initially considered as potentially influencing seed density (Table 1). Given the large set of explanatory variables, we first fitted univariate models to test individual parameters following the Wald test. We discarded all explanatory variables considering a *p* value cut-off point of 0.25 (Hosmer and Lemeshow 2000). Also, to ensure that highly correlated variables were not both used in the models, we tested for correlations using the Spearman's rank coefficient (Spearman 1907), and measured collinearity for all explanatory variables consisting of continuous data (threshold $r > 0.7$; Dormann et al. 2013). With the remaining set of explanatory variables, we fitted a multivariate model, including testing for interactions between variables. Model selection was performed through a backward stepwise procedure, starting from the complete model and the final model was selected based on the Akaike Information Criterion (AIC) (Akaike 1974). Finally, goodness-of-fit measures were estimated through Likelihood Ratio Tests and the residuals for the final fitted model were analyzed. Statistical analysis was performed with R 2.15.3 software, using the *glmmADMB* package (Fournier et al. 2012). Confidence level was set at 95% ($\alpha = 0.05$).

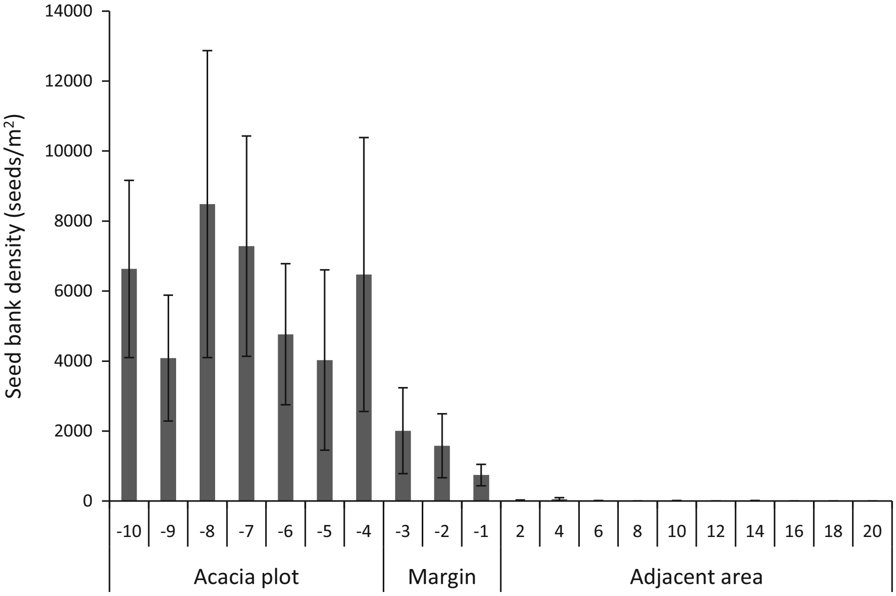
Results

Seed bank density

Exploratory analysis showed that the number of seeds was disproportionately higher in acacia plots than in the adjacent areas. In addition, it revealed that the number of seeds was higher in the center than in the margin of the acacia plots (Fig. 3).

On average, considering all sites, the number of seeds stored in the soil was significantly ($p < 2.2^{e-16}$)

Fig. 3 Number of seeds per square meter in acacia plots per distance class (from −10 m inside the plot to 20 m away from plot margin), averaged across sampling sites (A, B, C, D, and E). Bars represent mean ± standard error. $N = 15$



higher under the acacia plots (4608 seed/m^2 ± 820) than in the adjacent shrubby areas (9 seeds/m^2 ± 5). Outside the acacia plots, seeds were found up to 14 m away but only in six samples.

There were significant differences observed in seed bank density between the different acacia plots, with site A and D having more seeds than sites B, C, and E ($p = 1.95e^{-5}$), and sites B and E having less seeds than site C ($p = 0.02$). The highest average seed bank density was found in site A, where 12,507 seeds/m^2 (± 2781) were counted, while the lowest was registered in site B, with 319 seeds/m^2 (± 238). The maximum number of seeds collected was 62,747 seeds/m^2, in site A at −8 m (Fig. 4).

Statistical analysis revealed that inside the acacia plots seeds were not evenly distributed: the samples collected at the margins (samples −1, −2, and −3 m) had significantly fewer seeds (average 1444 seeds/m^2 ± 513) than the central ones (samples −4, −5, −6, −7, −8, −9, and −10 m; average 5965 seeds/m^2 ± 1127; $p < 2.2^{e-16}$).

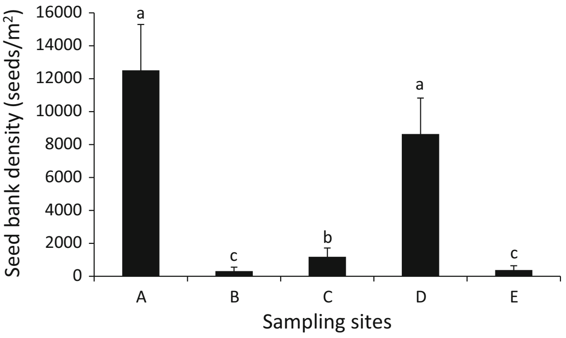


Fig. 4 Average number of seeds per square meter in the acacia plots of each sampling site (A, B, C, D, and E). Bars represent mean ± standard error. $N = 30$. Bars with the same letters are not significantly different

According to the univariate model, several variables contributed significantly to the differences in the seed bank density in the different study sites, the most significant being stoniness and time since last fire. Other variables, such as the number of fires occurring in the last 10 years, type of soil, *A. dealbata* trunk diameter, and density of trees, were also significantly related to seed bank density. Specifically, seed density increased with increasing time since last fire, trunk diameter and tree density; and decreased in sites with more stones and fires, southern exposure and shale soils (Table 2).

Although several variables contributed to the differences between sites, some of them were highly correlated and as such not all were included in the multivariate linear model. The final model included the four explanatory factors that together best explained the differences in the number of seeds found in each sampling site: stoniness, number of fires that had occurred in the last 10 years, and density of trees and roots (Table 3). Seed density decreased in areas that experienced a greater number of fires, and

Table 2 Univariate model results for the density of *A. dealbata* seed bank (significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Variables		Estimate	SE	*z* value	Pr(> |*z*|)
Exposure	South	−3.3238	1.4557	−2.283	0.0224*
Soil	Shale	−3.192	1.3377	−2.385	0.0171*
Trunk diameter		0.855	0.3764	2.272	0.0231*
Tree density		0.6669	0.314	2.124	0.0337*
Number of fires		−3.192	1.338	−2.385	0.0171*
Root density		−1928	1.88	−1.025	0.3053
Stoniness		−5.831	1.227	−4.752	$2.02e^{-06}$***
Time since last fire		0.54096	0.20745	2.608	0.00911**

Table 3 Final model obtained through the GLMM analysis for the density of *A. dealbata* seed bank (significance level: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Variable	Estimate	SE	*z* value	Pr(> |z|)
(Intercept)	10.4721	2.0763	5.044	$4.57e^{-07}$***
Tree density	0.6804	0.2117	3.213	0.0013**
Root density	−2.8622	0.8970	−3191	0.0014**
Stoniness	−2.8442	1.1072	−2.569	0.0102*
Number of fires	−2.6582	0.7201	−3.691	0.000223***

where higher stoniness and root density were observed; on the other hand, seed density increased with higher *A. dealbata* density.

Germinability and viability

Fewer seeds germinated without physical stimulus when incubated at 25 °C (first 30 days of the experiment) than seeds exposed to 60 °C for 10 h [69.0% (±4.6) vs. 8.6% (±3.8), respectively; $W = 1720.5$; $p = 1.493e^{-06}$]. Thirty days after physical stimulation significant differences were noted ($W = 706.5$; $p = 0.00668$), but in this case seeds exposed to a constant 25 °C temperature had higher germination than seeds kept at 60 °C for 10 h [81.1% (±2.9) vs. 14.1% (±8.2)]. In the end, between 83.4% ± 2.2 (for seeds incubated at 25 °C) and 89.7% ± 3.7 (for seeds incubated initially at 60 °C) of the seeds collected from the soil seed bank were viable, with only a small percentage of seeds decaying or being non-viable (Fig. 5).

When analyzing germination during the 2 months period (Fig. 6), a few seeds exposed to 25 °C germinated in the first 12 days, but most seeds germinated up to 4 days after scarification (day 30). On the other hand, seeds exposed initially to 60 °C germinated mostly in the first 6–12 days of the experiment, and 2 days after the physical stimulation there was a new germination peak (Fig. 6).

Discussion

In general, the density of *A. dealbata* seed bank observed in our study was lower than values referred by Gibson et al. (2011) either in Chile (10,000 seeds/m^2) or in Portugal (22,500 seeds/m^2). The values reported by these authors for Portugal refer to a site (Serra do Açor, Central Portugal) that had not burnt for more than 20 years (H Marchante et al. *unpublished data*) and as such the lower densities found in our study are probably explained by the occurrence of recent fires. These fires have probably depleted, at least partially, the seed bank that is known to be fire stimulated (Richardson and Kluge 2008) and as such the observed seeds have been accumulated only in the last 10 years of less (see discussion below). On the other hand, fires also imply that trees present are younger, producing fewer seeds, which results in lower densities of the seed bank. Additionally, we observed that soil was compacted in all the plots,

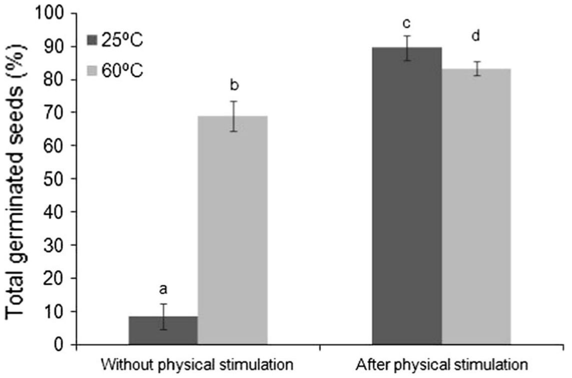


Fig. 5 Percentage of *A. dealbata* germinated seeds in two temperature treatments—constant exposure to 25 °C and initial exposure to 60 °C, with and without physical stimulation. Bars represent mean ± standard error. 25 °C $N = 29$, 60 °C $N = 36$. Bars with the same letters are not significantly different

which probably hindered seed infiltration into the soil, and subsequent accumulation in the seed bank.

The significant differences between sites, ranging from 319 seeds/m^2 to 12,507 seeds/m^2, may partially be explained by site characteristics. The lower numbers of seeds in sites with higher stoniness and root density may be explained because such features probably hinder seed infiltration in the soil. The number of seeds also decreased with increasing number of fires and with less time since last fire (time since last fire was more significant in the univariate model, while the number of fires was more influential in the multivariate model); accordingly, the seed bank density was higher in sites A and D and the last fire in both sites was more than 10 years ago. This was expected since fire is known to break seed dormancy of *Acacia* species, promoting massive germination and thus reducing soil seed bank size (Richardson and Kluge 2008). In Portugal, despite the lack of references, field observations suggest that *A. dealbata* first produces seeds at four to five years, similar to in its native range (Maslin and McDonald 2004), at least in some situations. Taking that into account, the occurrence of fires in older acacia plots every 4 years or less can considerably reduce the soil seed bank. Even so, mature individuals (i.e., flowering) and pods were found in every sampled site, even in sites B and C which burned in 2008, showing that in these sites seeds were produced between 2008 and 2012 (sampling was done during flowering period in February–April 2013). This suggests that either some adult trees survived the fire and keep producing seeds or sexual maturity was reached before plants reached 4 years of age (germination would probably have occurred only months after the fires, which occurred in July–September 2008, and fructification occurs in June–July in the northern hemisphere).

As expected, higher density of *A. dealbata* and bigger trees contributed to higher densities of seeds stored in the soil, and consequently higher seed density

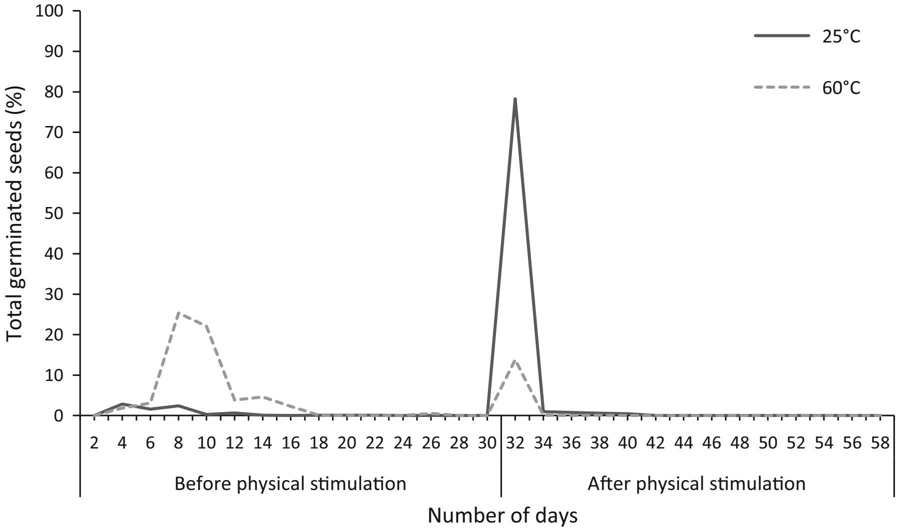


Fig. 6 Time course of seed germination after constant exposure to 25 °C and initial exposure to 60 °C, before and after physical stimulation

was found in the center of acacia plots and in areas that burned longer ago (longer-invaded areas).

In addition to the high number of seeds in the seed bank, it is important to stress that the seeds stored in the soil were highly viable (up to 89.7%). But, as expected, only a small portion germinated without stimulus (8.6%), confirming that seeds of this species remain dormant in the soil for a long time (Richardson and Kluge 2008). For many species, the break-down of seed dormancy can occur as a result of natural soil heating during the dry season or due to a brief exposure to intense heat during fires (Probert 2000). One of the most interesting results of our study was that the dormancy of *A. dealbata* seeds was effectively broken by exposure to 60 °C, with almost 70% of the seeds germinating without any other stimulation. In Portugal, temperatures of 49 °C have been registered at 5 cm above the soil surface in the study area (Horta 2014), and soil temperatures above 60 °C have been recorded 1-cm beneath soil surface in other areas of the Iberian Peninsula (Santana et al. 2013). This suggests that high temperatures (e.g., occurring during summer heat waves) can contribute to the germination of *A. dealbata* seeds, consequently explaining the expansion of the species in areas where no fire or other disturbances occur and where the species is present only in the seed bank. This needs to be taken into consideration in management programs, particularly when monitoring areas surrounding invaded plots. Although our sampling in areas adjacent to invaded plots was only exploratory and insufficient to fully quantify the seed bank outside the acacia plots, it clearly showed that *A. dealbata* seeds are present outside the acacia plots, possibly dispersed by ants (Richardson and Kluge 2008), winds or birds. Although the number of seeds found in adjacent areas was low and detected at a maximum distance of 14 m beyond the plot, seeds were viable which is enough to produce new foci of invasion. As the species has the ability (albeit low) to self-pollinate spontaneously (Correia et al. 2014) even if only one seed was found away from an acacia plot, this would be sufficient to start a new invasion.

Implications for management

In Portugal, control of invasive plants is often the result of isolated control programs, with limited funds and currently without a coordinated national plan. Control of *A. dealbata* is usually based on removing standing plants, either with or without stump application of herbicide, and sometimes debarking, often in small areas and leaving adult individuals in nearby areas (Fernandes 2008). In many cases, follow-up measures are not considered, both as a result of financial constraints or lack of knowledge, jeopardizing the initial control efforts and leading to massive seed germination and resprouting after clearing (Fernandes 2008; authors, *personal observation*). If successful control of *A. dealbata* is to be achieved, the prolific soil seed bank needs to be reduced, especially in large and long-invaded areas, but also where seeds are less numerous. Additionally, seeds found outside acacia plots emphasize the need to define follow-up and monitoring protocols that allow early detection of new individuals of the species (before tree maturity), not only inside the acacia plots but primarily in adjacent areas in order to contain expansion. Finally, the fact that seeds remain dormant for a long time (Richardson and Kluge 2008) highlights the need for follow-up measures to be assured for several years after the initial control is undertaken.

A number of methods have already been described for the management of *Acacia* species seed banks, including litter removal, earth covering, soil inversion, removal of the top soil, solarization, prescribed fire and biological control (Wilson et al. 2011). Biological control is considered one of the most cost effective, sustainable, and reliable long-term options (Adair 2008b; Marais et al. 2004; Marchante et al. 2011b; Wilson et al. 2011). In South Africa, the seed feeder *Melanterius maculatus* (Coleoptera) is being used for *A. dealbata* control (Adair 2008b; Wilson et al. 2011), and other agents are being tested. However, in Continental Europe, biological control for invasive plants has not been an option until recently when, after several years of testing and risk assessment procedures, an Australian gall wasp (*Trichilogaster acaciaelongifoliae*) was first released in 2015 to control *Acacia longifolia* (Marchante et al. 2017). Even so, the use of a biocontrol agent to control *A. dealbata* is not yet an option, as required testing is only now starting and needs to be completed and successful before any release occurs (H Marchante *personal communication*). Fire (prescribed or post wildfire-integrated interventions) may be an effective method to control the seed bank, but it needs to be carefully planned and assuring that adult trees are also removed (either mechanically or by fire), since the non-elimination of these implies constant renewal of the seed bank. Follow-up actions are also critical for the success of seed bank management, since it is extremely important to prevent re-invasion.

Conclusion

Acacia dealbata accumulates a large soil seed bank that is highly viable. Most seeds remain dormant in the soil, but 60 °C (attainable on very hot days or during heat waves) is enough to stimulate germination of a considerable part of the seed bank. Although seed banks are mostly concentrated under the canopy of the acacia trees, some seeds are found in adjacent areas, outside invaded areas. As a result, we stress the need to consider areas surrounding invaded plots in management plans, with particular attention not only after fires but also after heat events which may trigger germination. The seed bank functions as a hidden legacy: seeds can remain in the soil for a long time, with removal of adult trees being only a temporary solution. Our results highlight that the seed bank of species accumulating numerous and long-lived seeds need to be seen as a significant part of the invasive ability of the species, and must therefore be incorporated into management programs.

Acknowledgements The authors thank João Paula and José Paula for help in fieldwork and Maria João Janeiro for assistance with statistical analysis. Silvia Castro is acknowledged for useful comments and suggestions.

References

Adair RJ (2008a) Galling guilds associated with *Acacia dealbata* and factors guiding selection of potential biological control agents. In: Julien MH, Sforza R, Bon MC, Evans HC, Hatcher P E, Hinz HL, Rector BG (eds) Proceedings of the XII International Symposium on Biological Control of Weeds. La Grande Motte, France, pp 122–128, 22–27 Apr 2007

Adair RJ (2008b) Biological control of Australian native plants, in Australia, with an emphasis on acacias. Muelleria 26(1):67–78

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Contr 19(6):716–723

Andreu J, Vilà M (2007) Análisis de la gestión de las plantas exóticas en los espacios naturales españoles. Ecosistemas 16(3):109–124

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MH, White JS (2008) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evolut 24(3):127–135

Bradstock RA, Auld TD (1995) Soil temperatures during experimental bushfires in relation to fire intensity: consequences for legume germination and fire management in south-eastern Australia. J Appl Ecol 32:76–84

Correia M, Castro S, Ferrero V, Crisóstomo JA, Rodríguez-Echeverría S (2014) Reproductive biology and success of invasive Australian acacias in Portugal. Bot J Linnean Soc 174:574–588

Crisóstomo JA, Freitas H, Rodríguez-Echeverría S (2007) Relative growth rates of three woody legumes: implications in the process of ecological invasion. Web Ecol 7:22–26

D'Antonio C, Meyerson LA (2002) Exotic plant species as problems and solutions in ecological restoration: a synthesis. Restor Ecol 10(4):703–713

DAISIE European Invasive Alien Species Gateway (2006) *Acacia dealbata*. http://www.europe-aliens.org/speciesFactsheet.do?speciesId=12749 Accessed 11 Jan 2014

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, García Marquéz JR, Gruber B, Lafourcade B, Leitão PJ, Münkemüller T, McClean C, Osborne PE, Reineking B, Schröder B, Skidmore AK, Zurell D, Lautenbach S (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46

Fernandes MM (2008) Recuperação Ecológica de Áreas Invadidas por *Acacia dealbata* Link no Vale do Rio Gerês: Um Trabalho de Sísifo? Master Dissertation, University of Trás-os-Montes e Alto Douro, Portugal

Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder M, Nielsen A, Sibert J (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim Methods Softw 27:233–249

Fuentes-Ramirez A, Pauchard A, Lohengrin AC, García RA (2011) Survival and growth of *Acacia dealbata* vs native trees across an invasion front in south-central Chile. For Ecol Manag 261:1003–1009

Gibson MR, Richardson DM, Marchante E, Marchante H, Rodger JG, Stone GN, Byrne M, Fuentez-Ramírez A, George N, Harris C, Johnson SD, Le Roux JL, Miller JT, Murphy DJ, Pauw A, Prescott MN, Wandrag EM, Wilson JRU (2011) Reproductive biology of Australian acacias: important mediator of invasiveness? Divers Distrib 17:911–933

Gioria M, Pyšek P, Moravcová L (2012) Soil seed banks in plant invasions: promoting species invasiveness and long-term impact on plant community dynamics. Preslia 84:327–350

Horta MC (2014) Dados climáticos referentes a 2013—Posto meteorológico da Escola Superior Agrária. Instituto Superior de Castelo Branco, Portugal

Hosmer DW, Lemeshow S (2000) Applied logistic regression, 2nd edn. John Wiley & Sons Inc, Hoboken, p 375

ICN (2006). Plano Sectorial da Rede Natura 2000. Flora. Instituto da Conservação da Natureza, Direcção de Serviços da Conservação da Natureza, Lisboa

Le Maitre DC, Gaertner M, Marchante E, Ens EJ, Holmes PM, Pauchard A, O'Farrell PJ, Rogers AM, Blanchard R, Blignaut J, Richardson DM (2011) Impacts of invasive

Australian acacias: implications for management and restoration. Divers Distrib 17:1015–1029

Lorenzo P, Gonzáles L, Reigosa MJ (2010) The genus *Acacia* as invader: the characteristic case of *Acacia dealbata* Link in Europe. Ann For Sci 67:101

Lorenzo P, Pazos-Malvido E, Rubido-Bará M, Reigosa MJ, González L (2012) Invasion by the leguminous tree *Acacia dealbata* (Mimosaceae) reduces the native understory plant species in different communities. Aust J Bot 60:669–675

Marais C, van Wilgen BW, Stevens D (2004) The clearing of invasive alien plants in South Africa: a preliminary assessment of costs and progress. S Afr J Sci 100:97–103

Marchante H, Freitas H, Hoffmann JH (2011a) Post-clearing recovery of coastal dunes invaded by *Acacia longifolia*: is duration of invasion relevant for management success? J Appl Ecol 48(5):1295–1304

Marchante H, Freitas H, Hoffmann JH (2011b) Assessing the suitability and safety of a well-known bud-galling wasp, *Trichilogaster acaciaelongifoliae*, for biological control of *Acacia longifolia* in Portugal. Biol Control 56:193–201

Marchante H, Morais M, Freitas H, Marchante E (2014) Guia prático para a identificação de Plantas Invasoras em Portugal. Imprensa da Universidade de Coimbra, Coimbra, p 207

Marchante H, López-Núñez FA, Freitas H, Hoffmann JH, Impson F, Marchante E (2017) First report of the establishment of the biocontrol agent *Trichilogaster acaciae-longifoliae* for control of invasive *Acacia longifolia* in Portugal. EPPO Bull. doi:10.1111/epp.12373

Maslin BR, McDonald MW (2004) AcaciaSearch: evaluation of Acacia as a woody crop option for Southern Australia. Rural Industries Research and Development Corporation, Barton, ACT (RIRDC publication no. 03/017). ISBN 0642585857

McConnachie MM, Cowling RM, van Wilgen BW, McConnachie DA (2012) Evaluating the cost-effectiveness of invasive alien plant clearing: a case study from South Africa. Biol Conserv 155:128–135

Meireles C, Mendes P, Vila-Viçosa C, Cano-Carmona E, Pinto-Gomes C (2013) Geobotanical aspects of *Cytisus oromediterraneus* and *Genista cinerascens* in Serra da Estrela (Portugal). Plant Sociol 50(1):23–31

Ooi MKJ, Auld TD, Denham AJ (2012) Projected soil temperature increase and seed dormancy response along an altitudinal gradient: implications for seed bank persistence under climate change. Plant Soil 353:289–303

Ooi MKJ, Denham JA, Santana VM, Auld TD (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. Ecol Evol 4(5):656–671

Probert RJ (2000) The role of temperature in the regulation of seed dormancy and germination. In: Fenner M (ed) Seeds: the ecology of regeneration in plant communities, 2nd edn. CABI Publishing, New York, pp 261–271

Richardson DM, Kluge R (2008) Seed banks of invasive Australian *Acacia* species in South Africa: role in invasiveness and options for management. Perspect Plant Ecol Evol Syst 10:161–177

Richardson DM, Rejmánek M (2011) Trees and shrubs as invasive alien species—a global review. Divers Distrib 17:788–809

Richardson DM, Pyšek P, Simberloff D, Rejmánek M, Mader AD (2008) Biological invasions—the widening debate: a response to Charles Warren. Prog Hum Geogr 32:295–298

Rodríguez-Echeverría S, Afonso C, Correia M, Lorenzo P, Roiloa SR (2013) The effect of soil legacy on competition and invasion by *Acacia dealbata* Link. Plant Ecol 214(9):1139–1146

Saharjo BH, Watanabe H (1997) The effects of fire on the germination of *Acacia mangium* in a plantation in South Sumatra, Indonesia. Commonw For Rev 76:128–131

Santana VM, Bradstock RA, Ooi MKJ, Denham AJ, Auld TD, Baeza MJ (2010) Effects of soil temperature regimes after fire on seed dormancy and germination in six Australian Fabaceae species. Aust J Bot 58:539–545

Santana VM, Baeza MJ, Blanes MC (2013) Clarifying the role of fire heat and daily temperature fluctuations as germination cues for Mediterranean Basin obligate seeders. Ann Bot 111:127–134

Sanz-Elorza M, Dana E, Sobrino E (eds) (2004) Atlas de las plantas alóctonas invasoras en España. Dirección General para la Biodiversidad, Madrid, p 384

Spearman C (1907) Demonstration of formulæ for true measurement of correlation. Am J Psychol 18(2):161–169

Stoof CR, Moore D, Fernandes PM, Stoorvogel JJ, Fernandes RES, Ferreira AJD, Ritsema CJ (2013) Hot fire, cool soil. Geophys Res Lett 40:1534–1539

Vilà M, Espinar J, Hejda M, Hulme P, Jarošík V, Maron J, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett 14:702–708

Wijayabandara SMKH, Jayasuriya KMGG, Jayasinghe JLDHC (2013) Seed dormancy, storage behavior and germination of an exotic invasive species, Lantana camara L. (Verbenaceae). Int Res J Biol Sci 2(1):7–14

Wilson JRU, Gairifo C, Gibson MR, Arianoutsou M, Bakar BB, Baret S, Celesti-Grapow DiTomaso JM, Dufour-Dror JM, Kueffer C, Kull CA, Hoffmann JH, Impson FAC, Loope LL, Marchante E, Marchante H, Moore JL, Murphy DJ, Tassin J, Witt A, Zenni R, Richardson DM (2011) Risk assessment, eradication, and biological control: global efforts to limit Australian acacia invasions. Divers Distrib 17:1030–1046