Universidade de Aveiro Departamento de Biologia
Ano 2014

Isabel Maria Melo Mendes Passos

Importance of seed bank for the management of invasive *Acacia dealbata*

A importância do banco de sementes na gestão de *Acacia dealbata*

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica da Doutora Elizabete Maria Duarte Canas Marchante (Professora Auxiliar convidada) do Departamento de Ciências da Vida da Universidade de Coimbra e co-orientação da Mestre Rosa Maria Ferreira Pinho (Assessora) do Departamento de Biologia da Universidade de Aveiro

Aos meus Joões. Ao meu avô Tó.

o júri

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palavras-chave

Acacia dealbata, banco de sementes, germinabilidade, viabilidade, gestão.

resumo

O controlo de plantas invasoras é uma tarefa árdua, dificultada pela existência de bancos de sementes extensos e com longa viabilidade. A espécie *Acacia dealbata* é uma árvore nativa da Austrália, considerada como uma das espécies invasoras mais agressivas em Portugal. Sabe-se que o seu banco de sementes permanece viável no solo por muitos anos, no entanto a sua extensão e a viabilidade das sementes não são ainda conhecidos. O presente trabalho tem como objetivo caracterizar esse banco de sementes, a sua extensão, viabilidade e germinabilidade, de forma a contribuir para a sua gestão e para o conhecimento geral sobre a ecologia da espécie. A extensão do banco de sementes foi avaliada através da recolha de amostras de solo em áreas invadidas e zonas adjacentes não invadidas, realizando-se depois a contagem das sementes. Depois de contabilizadas, as sementes foram postas a germinar a 25ºC, sendo que uma parte foi exposta a uma temperatura inicial de 60ºC, com o objetivo de perceber se temperaturas extremas no solo podem quebrar a dormência das sementes. Os resultados indicam que nas áreas invadidas o banco de sementes tem uma densidade média de 4 608 sementes/m^2 (± 820), sendo que os valores máximos observados foram de 62 747 sementes/m^2. Nas áreas adjacentes observaram-se em média 9 sementes/m^2 (± 5), com um valor máximo de 632 sementes/m^2. Apesar de apenas 8,6% das sementes terem germinado sem qualquer estímulo, 89,7% do banco de sementes germinou após a escarificação das sementes, indicando que 81% das sementes armazenadas no solo se encontram dormentes. Das sementes inicialmente expostas a 60ºC, cerca de 64% germinaram sem escarificação, sugerindo que a ocorrência de temperaturas anormalmente altas pode estimular a germinação. De acordo com a análise estatística efetuada a probabilidade o número de sementes acumuladas no solo diminui com o aumento de pedregosidade e com a densidade de raízes; por outro lado, o banco de sementes aumenta com densidade de *A. dealbata* e em áreas sujeitas a menos incêndios. Os resultados obtidos demonstram a elevada viabilidade e densidade do banco de sementes de *A.dealbata*, evidenciando o elevado risco de reinvasão de áreas onde se realizam ações de controlo da espécie. Este estudo alerta para a necessidade de incluir a gestão do banco de sementes nos planos de controlo de espécies invasoras,alertando para o facto de a espécie ser capaz de dispersar sementes atém das áreas invadidas. Estas características transformam o banco de sementes numa herança escondida, que pode dificultar as ações de controlo. São discutidos diferentes métodos possíveis para lidar com bancos de sementes.

keywords *Acacia dealbata*, seed bank, seed germinability, seed viability, management.

abstract

The management of invasive plants is a hard task, which may be further hampered by the existence of extensive and long-lived seed banks. *Acacia dealbata* is an Australian tree considered one of the most aggressive invasive plant species in Portugal; the species is known to produce a persistent seed bank, but its extension and viability hasn't been explored. In this work we aim to characterize the seed bank of *A. dealbata*, namely its extension, germinability and viability, in order to contribute to the management of the species, but also increase knowledge about its ecology. The extension of the seed bank was assessed by collecting soil samples in invaded stands and adjacent areas and counting the seeds. These were then germinated at 25ºC and some were exposed to an initial temperature of 60ºC, aiming to assess the effect of extreme soil temperature in dormancy breaking. Results showed that beneath canopy the seed bank averaged 4 608 seed/m^2 (± 820), but maximum values can reach 62 747 seeds/m^2. In areas adjacent to the invaded stands a mean value of 9 seeds/m^2 (± 5) was found, with maximum values of 632 seeds/m^2. Although only 8,6% of the seeds germinated without any stimulus, in total 89,7% of the seeds found showed to be viable after scarification, with 81% of the seeds remaining dormant in the soil. Of the seeds exposed to 60ºC almost 64% germinated without any physical stimulation, suggesting that extreme soil temperatures, reached in hot days, may break seed dormancy. Multivariate analysis showed that the probability of accumulating high numbers of seeds in soil decreases with higher stoniness and root density, and increases with higher *A. dealbata* density and with fewer fire events. Our results show that the soil seed bank of *A. dealbata* is numerous and viable, suggesting that risk of re-invasion after plant removal is very high. It was also shown that the species has the ability to disperse seed beyond invaded stands Although frequently disregarded, this study alerts for the need to include extensive and long-lived seed banks in the management of invasive plants, since these hidden legacies may quickly hamper control efforts. Alternatives to deal with such seed banks are discussed.

Table of contents

List of figures

List of tables

1. General Introduction

Biological invasions have been receiving increasing attention in the past years (Richardson and Pysek, 2006; Richardson *et al.*, 2008), as worldwide invasive species have been considered as one of the major threats to biodiversity (Richardson *et al.*, 2008; McGeoch *et al.*, 2010; Vilà *et al.*, 2011), health, society and economy (Andreu & Vilà, 2007). This problem has grown in the last decades, with the increasing of intentional and unintentional introduction of alien species in all areas of the globe (McNeely, 2001; Del Monte & Aguado, 2003). According to Delivering Alien Invasive Species In Europe (DAISIE) there are 6658 terrestrial plant species which have been classified as alien in some part of Europe (http://www.europe-aliens.org/default.do). In Portugal 667 alien species are listed (Almeida & Freitas, 2012), but only a small part of them are currently classified as invasive. Portuguese legislation recognizes 29 invasive plants, which lists alien species and forbids the use and commercialization those that are identified as invasive (Ministério do Ambiente, 1999). However, the list of species considered as invasive needs to be updated as several problematic invasive species are not yet included (Marchante, 2011a)

Introduction of alien species has been happening for centuries (Richardson and Pysek, 2006) but the number of species transferred around the world has increased due to the higher frequency of international travel and trade (Alpert, 2006). However not all exotic species are invasive. Most of them play an important part in agriculture, landscaping and forestry production without causing problems. Many of these alien species become **casuals** in nature, as they can be found outside their cultivation site, were they can flourish and even reproduce but they do not form self sustainable populations without human aid and so rely on repeated introduction for their persistence. From these, a part manages to become **naturalized**: they overcome environmental barriers and reproduce consistently, forming self replacing populations for several generations without (or despite) human intervention. Naturalized species often recruit offspring freely, usually close to adult plants but do not necessarily invade natural, seminatural or human-made ecosystems. A small group of naturalized plants are capable to recruit offsprings at considerable distances from parent plants and in considerable numbers, spreading into areas away from sites of introduction and are thus considered **invasive**. They produce offsprings by seeds and other propagules, often in very large number, and thus have the potential to spread over a considerable area (Richardson *et al.*, 2000).

The mechanism that makes a naturalized plant become invasive can be related to several causes, changes or triggers, which enables the species to rapidly increase their distribution. These causes

can be natural or human related, as storms, fires, changes in land use, climatic change events, adaptation to a seed disperser or even the control of another invasive species that opens up space to colonization (Marchante, 2008b). After this stimulus, the invasive species increases its distribution area and sometimes it can modify the character, condition, form or nature of the invaded ecosystems causing long lasting changes – these species are called **transformer** species (Richardson *et al.*, 2000).

There are several features that may allow an alien plant to reproduce outside its native range without any human aid and become invasive. Most invaders shows at least one of the following characteristics: rapid growth rates, non specific pollinators, highly attractive flowers to pollinators, absence of natural enemies in the invaded range, habitat generalists, vegetative reproduction, efficient seed dispersal systems, production of allelopathic chemicals or prolific seed production (Rejmanek and Richardson, 1996; Sanz Elorza *et al.*, 2004; Richardson & Kluge, 2008; Lorenzo *et al.*, 2010a; Gibson *et al*., 2011). The last one may lead to the accumulation of extensive seed banks in the soil that can often stay viable for a long time, in some cases over 50 years (Richardson & Kluge, 2008; Gibson *et al*., 2011; Gioria *et al.*, 2012; Wijayabandara *et al.*, 2013).

Impacts of invasive plants on biodiversity can occur at ecosystem (e.g. soil nutrient levels, soil pH, litter decomposition rates, hydrological cycle), community (e.g. production, abundance, diversity and behavior) and species level (e.g. fitness and growth) (Fuentes-Ramírez *et al*., 2010; Vilà *et al.*, 2011). Many of these impacts cause long-lasting changes in ecosystem structure that tend to persist after the removal of the invasive species – called 'Legacy effects'. These effects may prevent or hinder the establishment of native species long after the removal of the invasive species (D'Antonio & Meyerson, 2002; Marchante *et al.*, 2008b; Lorenzo *et al.*, 2010c, Marchante *et al.*, 2011b).

The control and management of invasive species can be a very difficult and expensive task (D'Antonio & Meyerson, 2002; Andreu and Vilà, 2007). Scalera (2010) estimates that from 1992 to 2006 Europe has spent over 132 million Euros financing 300 RTD Framework Programmes and LIFE projects related to invasive species management. In Spain, Andreu and Vilà (2007) estimate that over 50 million Euros have been spent in invasive plant species control, between 1997 and 2007. In Portugal, to my knowledge, there is no accurate estimate of costs of invasive plants control, but considering only LIFE projects and a few other projects more than 6 million Euros

have been spent in the last decade, but it is clearly underestimated (E. Marchante, personal communication).

The methods used to control invasive plants include i) mechanical, ii) chemical and iii) biological methods. Frequently, the best approach is considered to be a combination of several control methods, including restoration programs (Richardson & Kluge, 2008; Wilson *et al.*, 2011). However, frequently management programs are based on the simple approach of removing the existing invasive stands, using mechanical and/or chemical methods, and limit or prevent their regeneration for a period of time (Le Maitre *et al*., 2011). This approach often fails to achieve the desired outcome of a functional ecosystem dominated by native species (D'Antonio & Meyerson, 2002; Jasson, 2005; Le Maitre *et al*., 2011; Marchante, 2011a), because most of the alterations caused by invasive species persist in time after their removal, limiting the recovery of natural vegetation. When long term follow up is not performed after the initial removal of invasive stands, the area is frequently invaded again. This is particularly problematic when dealing with species that have very large and long-lived seed banks (e.g. *Acacia* spp.) because seedlings emerge after control actions (Paynter & Flanagan, 2004; Marais *et al.*, 2004; McConnachie *et al.*, 2012), threatening the success of management programs (Richardson & Kluge, 2008; Gaertner *et al.*, 2009; Lorenzo *et al.*, 2010b; Le Maitre *et al.*, 2011). In this context, the control of invasive plants requires long-term investment.

Besides the great number of invasive seeds produced and stored in the soil, invasive plants can modify the native seed bank composition since they reduce the presence of native species, resulting in direct changes in seed rain (Gioria *et al.*, 2012). Changes in seed bank can also be due other factors, such as changes in the biotic and abiotic conditions that affect both recruitment and seed mortality rates, alteration in seed germination rates (Facelli, 1994) and, as years goes by, depletion of existing native seed bank (Gioria & Osborne, 2010, Gioria *et al.*, 2011). This represents a great problem when the goal is to restore native vegetation in previously invaded areas (Richardson & Kluge, 2008), although some long invaded areas can still hold the capacity to recover native functional plant communities (Marchante *et al*., 2011b). Nevertheless, active restoration of the sites using native species can be done, to hasten the process of colonization of managed areas by native species and minimize the possibility of reinvasion (Davis *et al.*, 2000).

In Portugal, *Acacia* species, native from Australia, are among the most aggressive invasive plant species (Marchante *et al*., 2008a; Marchante, 2008b; Fernandes, 2012). Almeida & Freitas (2006) lists 14 acacia species in Portugal, from which 8 are recognized as invasive by portuguese law (see

Table 1). Some key traits of *Acacia* species are responsible by many changes caused in the environment and gives them the ability to out-compete native plants: rapid growth rates, their high biomass accumulation, release of allelopathic compounds (Lorenzo *et al.*, 2010c), large and persistent seed banks (Richardson & Kluge, 2008; Marchante, 2011a) and the capacity to fix nitrogen (Yelenik et *al*., 2004;Rodríguez-Ecreverría *et al*., 2009). All these features allow them to transform the ecosystem, making *Acacia* spp. transformer species, limiting the growth and post clearing recovery of native vegetation (Marchante *et al*., 2011b). Among the different *Acacia* species invasive in Portugal, *Acacia dealbata* is considered one of the most aggressive invaders (Marchante *et al.*, 2005; Marchante *et al*., 2008a).

Table 1 – *Acacia* species present in Portugal (based on Almeida & Freitas 2006). Status based on Marchante *et al.*, 2008a, Marchante, 2008b. * Species recognized as invasive by Portuguese law (Decreto-Lei n.º 565/99 de 21 de Dezembro).

Species	Status
Acacia baileyana F. Muell.	Casual
Acacia cultriformis A. Cunn. ex G. Don	Casual
Acacia cyclops A. Cunn. ex G. Don fil.	Invasive
Acacia dealbata Link*	Invasive*
Acacia decurrens (J.C. Wendl.) Willd.	Casual
Acacia karroo Hayne	Naturalized*
Acacia longifolia (Andrews) Willd.	Invasive*
Acacia mearnsii De Wild.	Invasive*
Acacia melanoxylon R. Br.	Invasive*
Acacia pycnantha Bentham	Invasive*
Acacia retinodes Schlecht.	Invasive*
Acacia saligna (Labill.) H.L. Wendl.	Invasive*
Acacia sophorae (Labill.) R. Br.	Invasive
Acacia verticillata (L' Hér.) Willd.	Naturalized

Acacia dealbata is a perennial species, fast growing tree from the Fabaceae family that can grow up to 30m height. This specie is native to Australia and it was introduced in several countries becoming invasive in several parts of Europe (Sanz Elorza *et al.*, 2004; Fagúndez & Barrada, 2007; Marchante *et al.*, 2008a; Lorenzo *et al.,* 2010a; Fernandes, 2012) south America (Fuentes-Ramírez

et al., 2011), north America, southern Africa, Indian Ocean islands, New Zealand and even Australia (Richardson & Rejmanek, 2011).

In Portugal it was introduced with ornamental purposes, as forest species and for soil fixation (Marchante *et al*, 2008a). In its natural range *A. dealbata* is present in several habitats such as forests, woodlands, grasslands and riparian ecosystems on a great variety of soils (Lorenzo, 2010a). In invaded areas it is capable of occupying a great variety of habitats in a great range of altitudes and soils types, from sand dunes, to forests and urban areas. In Portugal, it can be observed nearby human infra-structures and urban areas, and also in natural areas like natural parks and Natura 2000 sites (Fernandes, 2008; Marchante *et al*, 2008a) where it has become one of the major conservation problems, being classified has a transformer species (Marchante, 2008b). This species forms monoespecific stands, hindering the development of native vegetation and modifying communities structure (Lorenzo *et al.*, 2012). It makes a huge investment in flowering, displaying highly attractive flowers that allure local generalists' pollinators what contributes to its high reproductive success (Correia *et al*., 2014).

Considering the extensive areas invaded areas by *A. dealbata*, several management programs have already been implemented in Portugal for the control of this species. However they are mainly based on removing the existing plants, using mechanical or chemical methods and follow-up is often insufficient to deal with regeneration (from seeds and/or resprouting) and seed bank management is often disregarded. Consequently, successful long-term outputs was seldom achieved (Fernandes, 2008; personal observation).

Considering *A. dealbata* potential to accumulate long-lived seed banks, to design sustainable management programs it is essential to study its seed bank, namely the extension and factors that influence it. It is based on this assumption that this study was defined. In this context the main goal was to characterize and quantify the seed bank of *A. dealbata* in different invaded stands, to understand its implications in terms of management and future risk of (re)invasion. In addition, we also intend to assess the viability and germinability of stored seeds, since only viable seeds can confer the species the ability to reinvade. Furthermore, as viable seed can be dormant, we aimed to understand if temperatures of hot days or heat waves (around 60ºC) are enough to stimulate seed germination, as this can trigger invasions without the need of further disturbances.

2. The importance of (long lived) seed banks for the management of invasive plants: the case study of *Acacia dealbata* Link. [(1)]

2.1 Introduction

Invasive species have been considered as one of the major threats to biodiversity (Richardson *et al.*, 2008; McGeoch *et al.*, 2010; Vilà *et al*., 2011), health, society and economy worldwide (Andreu & Vilà, 2007).

Prolific seed production is one of the features that allow alien plants to reproduce outside their native range without any human aid and become invasive (Sanz Elorza *et al.*, 2004; Richardson & Kluge, 2008; Gibson *et al*., 2011). For species with long-lived seeds, this characteristic can lead to the accumulation of extensive seed banks in the soil that can remain as a "hidden legacy" for a long time (Richardson & Kluge, 2008; Gibson *et al*., 2011; Gioria *et al.*, 2012; Wijayabandara *et al.*, 2013) and eventually jeopardize management efforts (Richardson & Kluge, 2008; Gaertner *et al.*, 2009; Lorenzo *et al.*, 2010b; Le Maitre *et al.*, 2011; Marchante, 2011a).

In spite of considerable efforts to manage and control invasive plant species, many management programs are based on the simple approach of removing the existing stands, using mechanical and/or chemical methods, and control their regeneration (Le Maitre *et al*., 2011). This approach often fails to achieve the desired outcome of a functional ecosystem dominated by native species (D'Antonio & Meyerson, 2002; Jasson, 2005; Le Maitre *et al*., 2011; Marchante *et al.*, 2011b), due to invasive species seedlings emergence after removal actions (Marais *et al.*, 2004; McConnachie *et al.*, 2012), what happens especially in species that have very large and long-lived seed banks (e.g. *Acacia* spp.). For these species, the success of a control plan can only be guaranteed if a significant reduction of the seed bank is achieved (Marchante, 2011a; Marchante *et al.*, 2011b; Gioria *et al.*, 2012).

In Portugal, one of the most aggressive invasive species is *Acacia dealbata* Link (silver wattle), which represents a major conservation problem (Marchante *et al.*, 2005; Fernandes, 2008; Correia *et al*., 2014). This is a perennial, fast growing tree from the Fabaceae family, which can grow up to 30m height. It is native to Australia and was introduced in different parts of the world becoming invasive in some parts of Europe (Sanz Elorza *et al.*, 2004; Fagúndez & Barrada, 2007; Marchante *et al.*, 2008a; Lorenzo *et al.*, 2010a; Fernandes, 2012), south America (Fuentes-Ramírez *et al.*, 2011), north America, southern Africa, Indian Ocean islands, New Zealand and even Australia (Richardson & Rejmanek, 2011). In its native range *A. dealbata* occurs in several habitats such as forests, woodlands, grasslands and riparian ecosystems and on a great variety of

soils (Maslin & McDonald, 2004; DAISIE (http://www.europe-aliens.org/default.do)). In the invaded range it is capable of occupy a vast altitudinal range and soil types and a great variety of habitats, from sand dunes to forests and urban areas (Sanz Elorza *et al.*, 2004; Fagúndez & Barrada, 2007; Marchante *et al.*, 2008a; Fuentes-Ramírez *et al.*, 2010; Lorenzo *et al.*, 2010b).

Like most *Acacia* species, *A. dealbata* produces a large number of seeds and a larger seed crop every 2–3 years (Maslin & McDonald, 2004; Richardson & Kluge, 2008). *Acacia dealbata* seeds are fire adapted and are probably dispersed by ants, *i.e*., myrmecochory (Richardson & Kluge, 2008). Published data about *A. dealbata* seed banks is scarce (Lorenzo *et al*., 2010a; Gioria *et al.*, 2012), but Gibson *et al*., (2011) refer scattered data of seed rain of 2 553 seeds/m^2/year, and a stored seed bank that ranges from 10 000 seeds/m^2 to approximately 22 500 seeds/m^2. Almost 80% of these seeds are stored within the first 10cm of soil (Richardson and Kluge, 2008) and can remain viable there for long periods of time - over 50 years – due to a water impermeability coat, which confers them the ability to remain dormant (physical dormancy) (Richardson & Kluge, 2008). This feature confers the species a very high potential for regeneration, especially in fire adapted habitats (Thompson & Grime, 1979), since germination of soil-stored seeds is triggered by heat (Adair, 2008b) associated with large diurnal temperature and fire (Fenner & Thompson, 2005), and by other disturbances (D'Antonio & Meyerson, 2002; Gibson *et al*., 2011; Le Maitre *et al.*, 2011). Even so, there are some records of *A. dealbata* present in sites where there are no signs of recent disturbances (Fuentes-Ramírez *et al*. 2011; Lorenzo *et al*., 2012).

Most wildfires can reach very high temperatures, which are known to break *Acacia* seeds dormancy (Richardson & Kluge, 2008; Wilson *et al.*, 2011). During these events, soil can reach temperatures from about 60ºC to over 800ºC (Stoof *et al.*, 2013). High soil temperatures reached during daytime in summer and heat waves are also documented to break seed dormancy in some species (Probert, 2000; Ooi *et al,* 2012b, 2014; Santana *et al.*, 2010, 2013). Some authors refer that 60ºC are enough to break the seed coat of *Acacia* species (Bradstock & Auld, 1995), while others state that these temperature are not enough to break dormancy of these species seeds (Saharjo & Watanabe, 1997; Ooi *et al,* 2014).

To control *A. dealbata* invasion, several management programs have been implemented in Portugal, usually removing the existing plants, using mechanical or chemical methods. However, successful control is seldom achieved because follow-up is often insufficient to deal with regeneration from seeds or resprouting, and seed bank management is disregarded (Fernandes, 2008; personal observation).

Considering the potential of *A. dealbata* to accumulate long-lived seed banks, in order to support a sustainable management of this species invasion it is essential to study its seed bank, namely the extension and factors that influence its density. In this context, the main goal of our work was to characterize and quantify the seed bank in different stands invaded by *A. dealbata*, and to understand its implications in terms of management and future risk of re-invasion. In addition, we also intend to assess the viability and germinability of stored seeds, since only viable seeds can contribute to reinvasion. Furthermore, as viable seeds can be dormant, we aim to understand if temperatures of hot days or heat waves (around 60ºC) are enough to stimulate seed germination, as this can trigger invasions without the need of further disturbances. As a secondary goal we also want to explore if there are seeds outside the invaded stands, since this is often ignored but in fact the existence of seeds in these areas can function as invasion focus and easily increase the invaded area.

2.2 Methods

This study took place in central Portugal, in Beira Interior region (Figure 1) where *A. dealbata* is present for a long time and where it is spreading rapidly in the last years. Nowadays it dominates large areas and new focus of invasion can be seen, some of them in the surroundings of important conservation areas, such as Natural Park of Serra da Estrela or Serra da Gardunha, where important populations of rare plant species and endemic plant communities are known (Pinto da Silva, 1956; ICN, 2006; Ribeiro *et al.*, 2012; Meireles *et al*., 2013) (Figure 1).

The study area has a typical Mediterranean climate with a large daily temperature amplitude. The annual average temperature varies between 11,2 °C in the north area (Weather station no. 082, Guarda, Lat.: 40°32'N; Lon.: 07°16'W, period 1981-2010) and 15,9 °C in the south (Weather station no. 507, Castelo Branco, Lat.: 39°50'N; Lon.: 07°28'W, period 1981-2010). It can rise up to a maximum temperatures of 41,6 °C in the summer (Castelo Branco) and a minimum temperature of -10.8 °C in the winter (Guarda). The annual average precipitation is 780 mm in the south and 920 mm in the north. The soils are acid, dominated by granite and schist and maximum soil temperature, at 5 cm deep, can reach up to 49ºC during summer (Horta, 2014).

Within the study area five sites were selected (A, B, C, D and E, Figure 1), based on several criteria: presence of only one *Acacia* species (*A. dealbata*), well individualized stands, stand size (at least 300 m^2), vegetation and size of the surrounding area (scrubby vegetation at least 20m around the invaded stand) and accessibility. Samples were collected in stands where *A. dealbata* cover

reached between 80 and 100%, but native plants were sometimes present in the understory. All sites burned in the last 10 years and consequently *A. dealbata* individuals present in these areas are not particularly big; this type of invaded area is very frequent in Portugal, due to the high frequency of fires. Fire is a constant element throughout the study area and all sites have burned in recent years, being the more recent a fire in 2008 in sites B and C (Table 2). Neighboring vegetation was predominantly shrubby, mostly dominated by *Cytisus* sp. There are no records or evidence of planting of *A. dealbata* in any of the study sites and as such it is assumed that the species spread by its own means to these areas. Several parameters were registered for each study site in order to explore the variables influencing the seed bank density (Table 2).

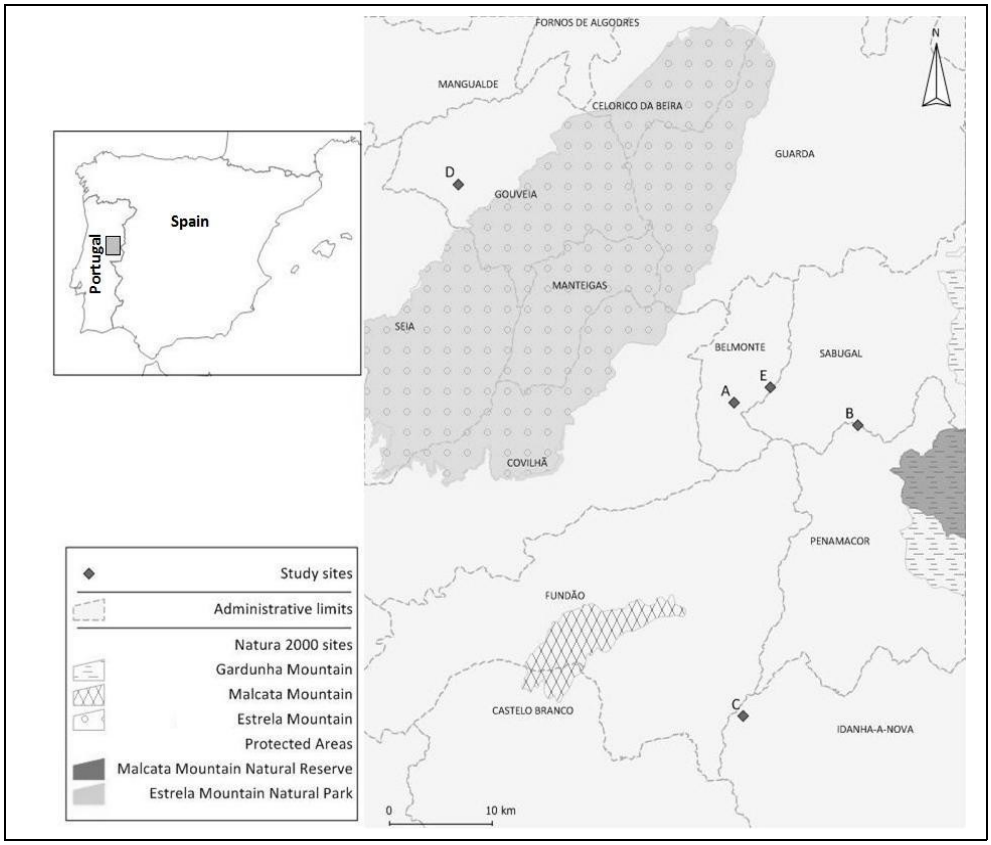


Figure 1 –Location of sampling sites (A,B,C,D,E) in Portugal

Sampling was conducted between February and April 2013, during flowering and before seed rain. In each sampling site three 30 meter long transects were set (Figure 2). The point under the first *A. dealbata* branch was considered the margin (0 meters). To evaluate the seed bank inside the stands 10 samples were collected, spaced 1 meter (samples designated as "-"). In adjacent

areas 10 soil samples were collected spaced 2 meters (samples designated as "+"), in order to access seed dispersion distance (Figure 2). Sampling in adjacent areas was not intensive, aiming only to detect the presence of seeds in these areas and not to characterize *A. dealbata* seed bank in non invaded areas; considering the extension and heterogeneity of such areas a full characterization of the seed bank of *A. dealbata* in such areas would imply a much greater sampling effort. In total, in each transect 20 samples were collected, making a total of 60 samples per site (30 inside the invaded area and 30 in adjacent areas). The first 3 samples collected inside the stand were considered as the margin of the stand (samples -1, -2, -3) and remaining were considered as the interior (-4 to -10) (Figure 2). There was always a distance of at least 20 meters between the samples collected in adjacent areas and *A. dealbata* individuals who did not belong to the sampled stands.

For each sample, the top 10 cm of soil, including leaf litter, were collected using an 11 cm diameter core. The samples were then dried and screened and the number of seeds was counted for each one of the samples.

Table 2 – Study sites characterization. Data was collected during sampling and used in the statistical analysis.

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 (º)	18	24	18	6	10
	Soil	Granite	Shale	Granite	Granite	Granite
Stand	**Average *Acacia dealbata* 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 (cm)	2,4	1,9	2,2	3,2	2,1
	Maximum trunk diameter (cm)	11,7	5,2	6,1	9,9	12,6

Variables		Site A	Site B	Site C	Site D	Site E
Sample*	***Acacia dealbata* fruit**	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 fires	1	2	1	1	1
	Cut**	1	0	0	0	1
	Garbage**	0	0	0	0	1

*A qualitative scale was used to classify each sample and the mean value of the stand was then calculated (0 - none; 1 - few; 2 - plenty). **it was considered only the presence (1) or absence (0).

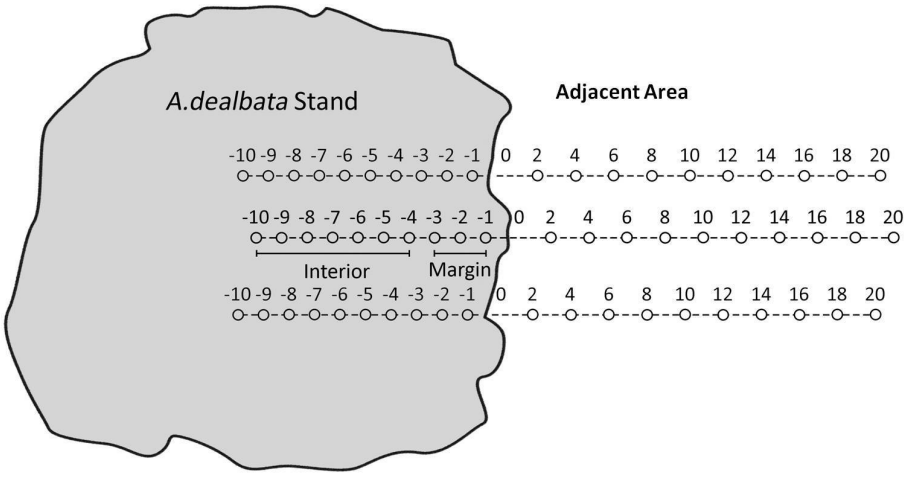


Figure 2 – Experimental set up of transects established to evaluate the seed bank, with 10 m lying inside the stand (0 to -10 m) and 20 m in the adjacent area (0 to 20 m); the point under the first *A. dealbata* branch was set as point zero. Samples were collected with 1 m interval under the canopy and 2 m interval outside the stand.

Seeds recovered from soil were checked for germinability and viability. A maximum of 50 seeds for sampling point was considered in cases where higher number of seeds was collected. Initially all the seeds were sterilized (Crisóstomo *et al.*, 2007). For germinability (seeds germinating without stimulus), part of the seeds were put at 25ºC to germinate with no further stimulus, for 30 days. To check the influence of extreme soil temperatures in germination, the rest of the seeds

were submitted to an initial temperature of 60ºC during 10 hours. After this initial heat shock, greenhouse temperature was set at 25ºC to allow normal germination. The seeds were checked out regularly, 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 scarified to test for viability (total of viable seeds (dormant or not)), during another 30 days. If after this period seeds had not germinated they were considered not viable. The total number of viable seeds was considered the sum of all germinated seed, with or without stimulus. The seeds at 25ºC that had not germinated were considered dormant.

Statistical analysis

An initial exploratory analysis was performed to observe the distribution of seeds along transects. This analysis suggested differences between the interior and the margin of the invaded stands and consequently the following analysis were performed considering these two areas.

To test if the number of seeds (density), germinability and viability was similar in the invaded stand and adjacent area, between the margin and the interior of the invaded area and between different temperature treatments a analysis of deviance (GLM) with Poisson distribution was used. Data in percentage were arcsine transformed.

Then, to explain differences in seed density between study sites, we used the generalized linear mixed models (GLMM) with a Poisson distribution. A set of 17 explanatory variables was considered (Table 2). Initially we tested the significance of each variable using a univariate model and predictor variables were those that had p-value less than 0.25. Afterwards a multivariate model was constructed with the predictor variables. To ensure that highly correlated variables were not used in the multivariate model, we tested correlations using the Spearman's rank coefficient (Spearman, 1907). The multivariate model variables were chosen through a backward stepwise procedure, 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% ($\alpha = 0.05$).

2.3 Results

Seed bank density

Exploratory analysis of the results showed that the number of seeds was disproportionately higher in the invaded stands than in the adjacent areas. In addition, it stressed out that seed numbers were higher in the interior than in the margin of the invaded stand (Figure 3).

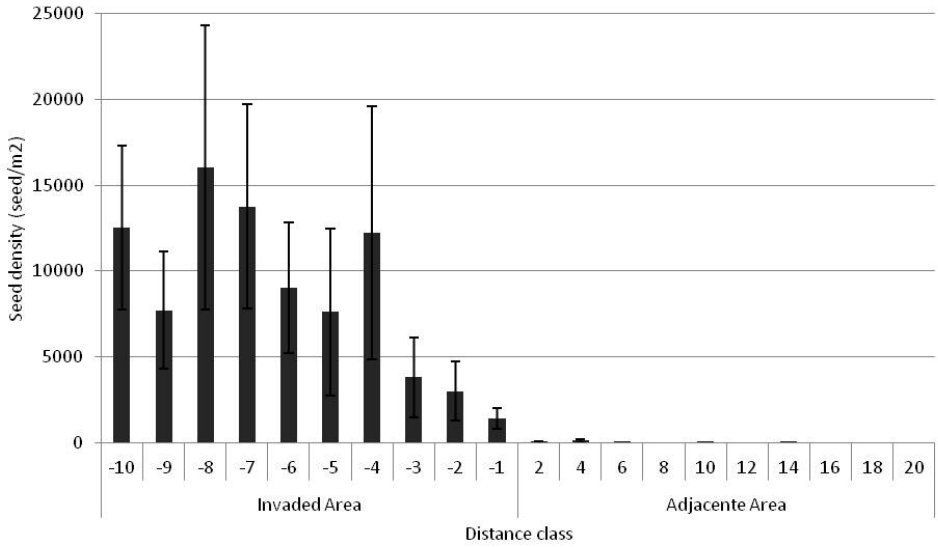


Figure 3 – Number of seeds per square meter in *A. dealbata* stands per distance class, considering all sampled stands (A, B, C, D and E). Bars represent the standard error. N=15.

In average, considering all sites, the number of seeds stored in soil was significantly ($p < 2.2^{e-16}$) higher inside the acacia stands (4 608 seed/m^2 ± 820) than in the adjacent shrubby areas (9 seeds/m^2 ±5). Outside the invaded areas seeds were only found up to 14 meters and only in a small number of samples – six in total.

Significant differences were observed in seed bank density of invaded areas between different sites, with site A and D having more seeds than sites B, C and E ($p= 1,95e^{-5}$, Figure 4) and sites B and E having less seeds than site C (p= 0,02, Figure 4). The highest average seed bank density was found in site A, where 12 507 seeds/m^2 (± 2 781) were counted while the lowest was registered in site B, where 319 seeds/m^2 (± 238) were found. The maximum number of seeds collected was 62 747 seeds/m^2, in site A at -8m.

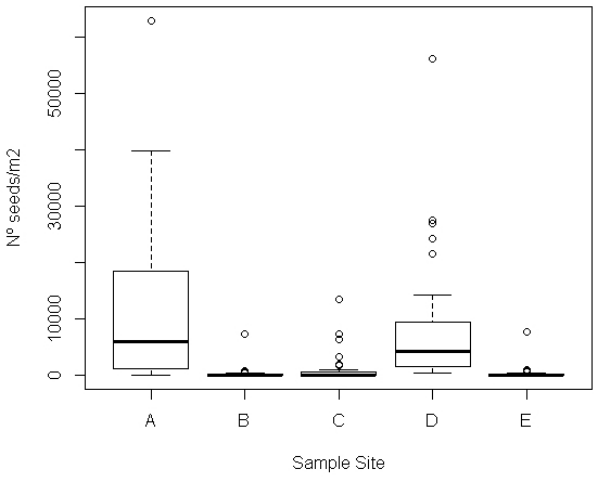


Figure 4 – Number of seeds per square meter in the invaded stands of each sampled site (A, B, C, D and E). The box represents 50% of sampled values, divided by the horizontal bar which represents the median. Outside bars represent minimum and maximum samples values. Dots correspond to possible outliers. N=30.

Statistical analysis revealed that inside the acacia stand seeds were not evenly distributed. Significant differences were found between the samples collected at the margins (samples -1, -2, -3m) and the interior ones (samples -4, -5, -6, -7, -8, -9, -10m), with the margins having significantly less seeds ($p < 2.2^{e-16}$, Figure 5).

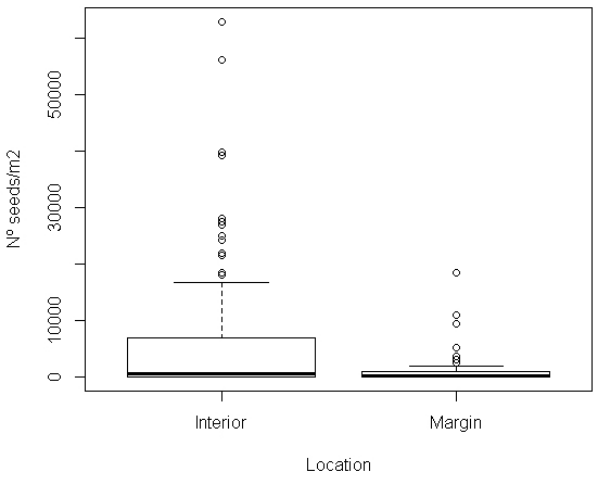


Figure 5 – Number of seeds per square meter in the interior and in the margins of *A. dealbata* stands, considering data from all sites. The box represents 50% of sampled values, divided by the horizontal bar which represents the median. Outside bars represent minimum and maximum samples values. Dots correspond to possible outliers. Margin N=45, Interior N=105.

According to the univariated model several variables contributed significantly to explain the differences in seed bank density in the different study sites, being the most significant stoniness and time since last fire. Other variables, such as the number of fires occurred in the last 10 years, type of soil, trunk diameter, and density of trees were also significantly related with seed bank density (Table 3).

Table 3 – Univariated model results for the extension of *A. dealbata* seed bank (significance level: * p<0,05, **p<0,01, ***p<0,001).

Variables		**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Exposure	South	-3,3238	1,4557	-2,283	0,0224*
Soil	Shale	-3,192	1,3377	-2,385	0,0171*
Average Trunk diameter		0,855	0,3764	2,272	0,0231*
Average *A. dealbata* density		0,6669	0,314	2,124	0,0337*
Number 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***
Last fire		0,54096	0,20745	2,608	0,00911**

Although several variables contribute to the differences between sites it was not possible to include them all in the multivariate linear model since they were highly correlated. The final model included the 4 explanatory factors that together better explained the differences in the number of seeds per square meter found in each sampling site: stoniness, number of fires occurred in the last ten years, tree and roots density ().

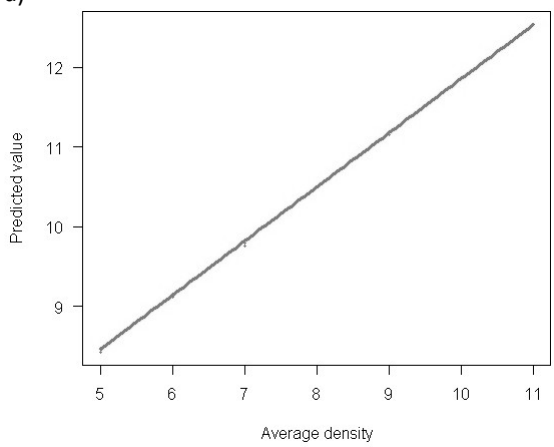
Table 4 – Final model obtained through the GLMM analysis for the number of seeds per square meter in the *A. dealbata* seed bank (significance level: * p<0,05, **p<0,01, ***p<0,001). Seed density decreases with higher number of fires occurred, sample stoniness and root density, it increases with higher *Acacia dealbata* density.

Variable	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	10,4721	2,0763	5,044	4,57e-07***

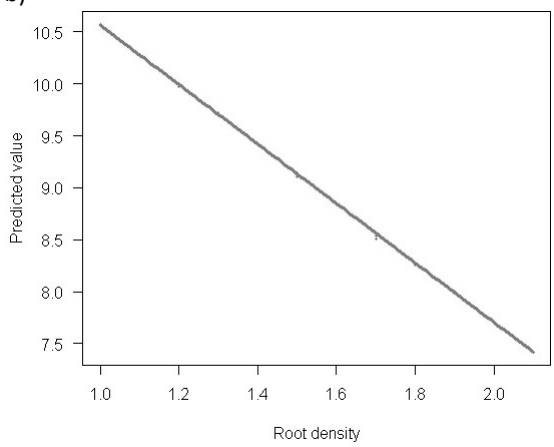
Variable	Estimate	Std. Error	Z value	Pr(>|z|)
Average *A. dealbata* density	0,6804	0,2117	3,213	0,0013**
Root density	-2,8622	0,8970	-3191	0,0014**
Stoniness	-2,8442	1,1072	-2,569	0,0102*
Number of fires occurred	-2,6582	0,7201	-3,691	0,000223***

The probability of having a higher number of seeds in soil was significantly higher in sites with higher *A. dealbata* density and with less fire events. Also, the number of seeds decreased when the samples had higher stoniness and root density (Figure 6).

a)



b)



c)

d)

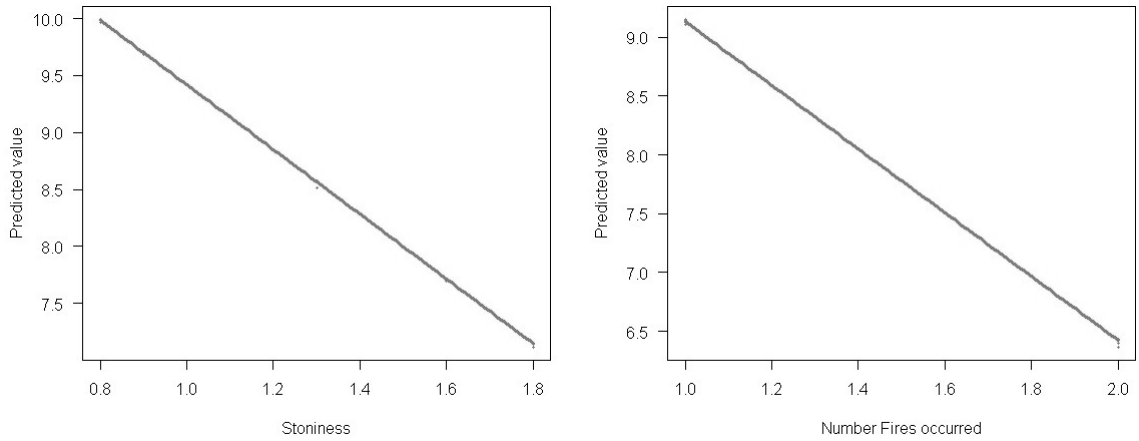


Figure 6 – Variables influence in *A. dealbata* seed density (seeds/m^2). Seed density increases with higher a) *A. dealbata* stand density, and decreases with higher sample b) root density, c) stoniness and d) number of occurred fires. N=15

Germinability and viability

After 30 days with no stimulus, only 8,6% (± 3,8) of the seeds maintained at 25 °C germinated while after physical stimulation (scarification) the percentage of germinated seed rose up to 89,7% (± 3,7), corresponding to the total viable seed bank (10,3% of the seed decay and were considered non viable). Considering the percentage of seeds germinated without stimulus and the number of rotten seeds, 81% (± 5) of *A. dealbata* seeds remains dormant in soil, accumulating in the seed bank.

For the seeds exposed to 60°C during 10 hours, 63,8% (± 6,1) germinated without any physical stimulation. After scarification, the percentage of germinated seeds rose up to 78% (± 6,8) (Figure 7).

The percentage of seeds germinated without scarification (first 30 days of the experiment) was significantly higher in seeds exposed to 60ºC than in seeds at 25ºC (p = 0,001). At the end of 60 days, *i.e.*, 30 days after physical stimulation was applied, no significant differences were found between the two treatments (W = 706.5; *p* =0,35), but in this case seed exposed to constant 25ºC temperature germinated more.

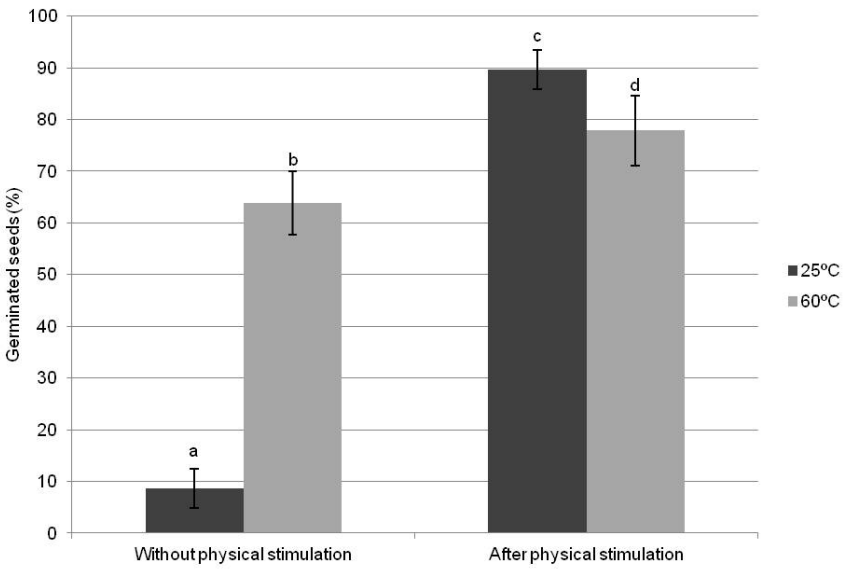


Figure 7 – Percentage of germinated seeds in the two different temperature treatments – constantly exposed to 25ºC and initial exposition to 60ºC, with and without physical stimulation. Bars represent standard error. 25ºC N=29, 60ºC N=75. Bars with the same letters are not significantly different.

The seeds exposed only to 25ºC germinated essentially after the physical stimulation took place, while seeds exposed to initial temperature of 60ºC had a greater germination percentage in the first 12 days of the experiment, when almost 60% of these seeds germinated. After the physical stimulation, these seeds had a new germination peak, leading to a total percentage of germinated seed of 78% (Figure 8).

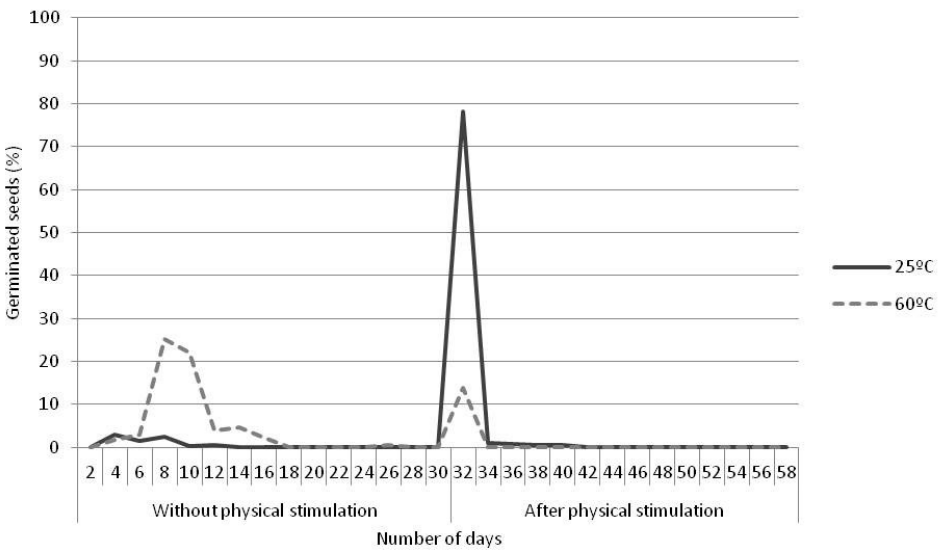


Figure 8 – Evolution of seed germination through time, in percentage.

2.4 Discussion

Although highly variable, our results for seed bank density of *A. dealbata* were in the same order of magnitude than those referred by Gibson *et al.* (2011) in Chile and in Portugal, where 10 000 seeds/m^2 and 22 500 seeds/m^2 were registered, respectively. The number reported for Portugal refers to a site that had not burn for more than 20 years (author personal observation) and as such the lower density found in our study is probably explained by the recent fires, which have probably depleted at least partially the seed bank and at the same time implies that trees are still small and producing fewer seeds, or by different soil characteristics (see discussion bellow). Moreover these values are higher then what has been described for other acacia species in Portugal, namely *Acacia longifolia* with a soil seed bank ranging between 500 and 1500 seeds/m^2 (Marchante, 2010).

The big differences between sites, ranging from 319 seeds/m^2 (± 238) in site B to 12 507 seeds/m^2 (± 2 781) in site A, may partially be explained by site characteristics. Stoniness, roots density, number of fires occurred and *A. dealbata* average density were the characteristics which together best explained the number of soil stored seeds. The first two influenced more the seed density, with the number of seeds decreasing with higher stoniness and roots density. During fieldwork we observed that soil was very compacted in all the stands, and sometimes root net was massive. This feature, along with the stone content, has probably hinder seed infiltration in the soil, contributing to a less dense seed bank.

Fire also influenced soil seed bank density, with the number of seeds decreasing with higher number of fires and with less time past since last fire. Although time since last fire was more significant in the univariate model, the number of fires was more influential in the multivariate model, and it is clear that two of the sites where the last fire was ten years ago (A and D) showed higher seed bank density. Fire is known to break seed dormancy in *Acacia* spp., promoting massive germination and thus great seed bank reduction (Richardson & Kluge, 2008). According to Maslin & McDonald (2004), *A. dealbata* first seed production happens at 4 to 5 years old in their native range. In Portugal, despite the lack of references, field observations indicate that it should be similar. Taking that into account, the occurrence of fires in older *A. dealbata* stand, every 4

years or less can significantly reduce the soil seed bank. Even so, mature individuals of large proportions and pods were found in every sampled site, even in sites B and C which burned in 2008. This suggests that in these sites seeds must have been produced between 2008 and 2012, before the new individuals supposedly reached sexual maturity and so some adult trees must have survived the fire and keep producing seeds.

As could easily be expected, higher *A. dealbata* density also contributed to a higher number of soil stored seeds. Also, seed density was higher in interior areas of invaded stands, where larger individuals were located, and so larger trees must be contributing more significantly to seed bank. Our results suggest that the seed bank of *A. dealbata* may be much larger in long-invaded areas where there have been no fires over the last years and where, consequently, the stands are denser and/or the size of trees is bigger.

In addition to the high number, our results also show that seeds of *A. dealbata* stored in the soil have high viability (89,7%), despite only a small part germinate without stimulus (8,6%), confirming that seed of *A. dealbata* may remain dormant in the soil for a long period of time (Richardson & Kluge, 2008), waiting for the right stimulus or condition to germinate. For many species, break-down of seed dormancy can occur as a result of natural soil heating during the dry season or of brief exposure to intense heat during fires (Probert, 2000). In our study we found that the dormancy of *A. dealbata* seeds was effectively broken by exposure to 60ºC, with almost 60% of the seeds germinated without any physical stimulation. Even so, soil seed bank is not completely depleted as part of the seeds are not stimulated at 60ºC and remain dormant in the soil. In Portugal, temperatures of 49ºC have been registered (Horta, 2014) 5cm above soil surface in the study area, and in the Iberian Peninsula soil temperatures above 60ºC have been recorded beneath soil surface (Santana *et al.*, 2010). This suggests, that heat waves can probably contribute to the germination of *A. dealbata* seeds, and consequently for the expansion of the species in areas were no fire or other disturbances have occurred and were the species is only present in the seed bank. This needs to be taken into consideration for management plans because as was shown by our results, seeds are present not only beneath *A. dealbata* canopy, but also outside the invaded stands. Although our sampling outside invaded stand was only exploratory and insufficient to fully quantify the seed bank in this area, it clearly showed that *A. dealbata* seeds can disperse and are present outside the invaded stands. The number of seeds found in adjacent areas was low and at the maximum distance was 14m beyond the stand, but seeds were there and were viable which is enough to create new foci's of invasion. In addition, since the species has

the ability (albeit low) to selfpollinate spontaneously (Correia *et al.,* 2014) even if only one seed was found away from the invaded stand, this is sufficient to start a new invasion focus.

The high density and viability of the seed bank together with the seeds dormancy, which allows the species to remain viable in soil for several years, and ability to disperse seeds far from the parent plant contribute to the success of *A. dealbata* as an invasive plant. These features make eradication of this species, even if in limited areas, probably out of reach. Nevertheless, management and control are feasibly and need to consider the seed bank; this will be discussed next.

Management implications of long-lived seed banks

In Portugal, invasive plants control is generally the result of isolated control programs, taken by private or public companies, or voluntary actions, with limited funds programs, without national planning. Control of *A. dealbata* is usually based on removing standing plants, either with or without stump application of herbicide, often in small areas and leaving adult individuals in nearby areas (Fernandes, 2008). In some cases follow up measures are not even considered, due to lack of money, obligation or knowledge, jeopardizing the initial control efforts due to massive seed germination and resprout after clearing (Fernandes, 2008).

If successful control of *A. dealbata* is to be achieved, the numerous soil seed bank shown in our study needs to be reduced, especially in large and long-invaded areas. Additionally, the seeds found outside the invaded areas alert for the need to define follow-up and monitoring protocols that allow early detecting of new individuals of the species, inside the invaded areas but also in adjacent areas. Furthermore, the fact that seeds remain dormant for a long time (Richardson & Kluge, 2008) highlights that follow-up measures need to be assured several years after the initial control is undertaken.

Several methods have been described for the management of *Acacia* spp. seed bank, including litter removal, earth covering, soil inversion, removal of the top soil, solarization, prescribed fire and biological control (Wilson *et al*., 2011). Excluding disturbance, prescribed fire and biological control, all these methods are difficult to apply in large areas, are highly destructive and are probably very expensive to implement, although they can be implemented in small areas (Wilson *et al*., 2011). Advantages and disadvantages of each method are summarized in Table 5.

Litter removal can be a good option in sensible areas, since it does not imply direct disturbances of the soil and thereby it will not affect significantly the native (or invasive) species seed bank. However it only implies the prevention of new seeds incorporation in the seed bank, not the management of existing seed bank.

Earth covering, soil inversion and removal of the top soil implicate major disturbances which are not suitable to sensible areas such as sand dunes, riparian areas or conservation areas. Moreover, earth covering implies the existence of enough soil to cover the managed area (Richardson and Kluge, 2008; Wilson *et al.*, 2011), implying other the disturbance of other areas.

Fire is a natural element of Mediterranean ecosystems. The occurrence of wildfires can be used to control invaded areas, being incorporated as part of a control program – post fire intervention. This approach would reduce drastically initial control costs; however it requires a great knowledge of recently burned areas (cartography - in order to establish priority targets) which is not always available.

Prescribed fire may also be a suitable tool, yet its use may be controversial, especially if control programs are implemented in sensible areas, where fire can destroy also other species seed banks, alter soil properties and promote erosion (DiTomaso *et al.,* 2006).

Other option is the use of biological control (Adair, 2008a; Wilson *et al.*, 2011), that is considered the most cost effective, sustainable and reliable option (Adair, 2008a; Marais *et al.*, 2004; Marchante *et al*., 2011c; Wilson *et al*., 2011). Depending on the agent used, this method may allow the depletion of the seed bank without causing disturbances and can be implemented in a wide and inaccessible area. Biological control of *A. dealbata* is currently being implemented in South Africa using the Coletoptera *Melanterius maculates* (Wilson *et al.*, 2011) who feeds on seeds (Adair, 2008a). In Portugal, biological control for invasive plants has never been used; therefore, this is not a suitable option at the moment.

Over all, none of the methods is totally effective on its own, and should be used with other methods as an integrated approach. They should not be separated from individual's removal, since the non elimination of adult individuals implies constant renewal of the seed bank. Follow up actions are also critical to the success of seed bank management, since it is extremely important to prevent re-invasion.

Thus, fire (prescribed or post wildfire intervention) seems to be the most effective method to control the seed bank, combined with the removal of adult individuals, but its use need to be

carefully planned. Additionally, biological control may have a crucial role to deplete the stored seed bank. Without the use of this tool, *A. dealbata* control will possibly take much longer, as seeds will continue to accumulate in the soil. Therefore, biocontrol safety tests needs to be addressed in Portugal, in order to enable its use.

Table 5 – Seed bank control methods. Advantages and disadvantages of each method. Based on Wilson *et al.*, 2011.

Method	Objective	Disadvantages	Advantages
Litter removal	Prevent some seeds to enter the seed bank	Difficult to implement in large areas; Follow up control actions are crucial to eliminate possible seedlings; Litter needs proper handling; Does not eliminate the problem, as seed bank is not directly affected; Measures are needed to stimulate seed bank germination.	Does not destroy other species seed bank; Maintenance of soil conditions and characteristics; Suitable use in sensitive areas.
Earth covering (10cm of soil)	Prevent seedling recruitment	Difficult to implement in large areas; Needs a greats amount of earth without invasive plant seeds and with similar characteristics to the intervened site; Possible introduction of plant species not typical/adapted to the intervened site; Need of post clearing of non typical/adapted plants; Does not eliminate the problem and buried dormant seeds can still become an issue.	Maintenance of good soil conditions (if used soil has similar characteristic to soil of managed area).
Soil inversion (10cm of soil)	Reduce seed numbers and seed survival	Difficult to implement in large areas; Does not eliminate the problem and buried dormant seeds can still become an issue.	No need of great amount of earth; Eliminates the possibility of

Method	Objective	Disadvantages	Advantages
			introducing species not typical/adapted to the intervened site.
Removal of the top 20cm	Reduce seed numbers	Difficult to implement in large areas; Removal of other species seed banks; Loss of the most fertile soil layer, which can difficult vegetation recovery; Removed soil needs proper handling; Doesn't eliminate the problem; Removed soil cannot be used in natural areas without treatment	No need of great amount of earth. Eliminates the possibility of introducing species not typical/adapted to the intervened site.
Solarization	Induce seed germination and seedling elimination	Difficult to implement in large areas; Plastic sheets are easily removed or damage; Destruction of other species seed banks/seedlings.	Easy to implement in very localized areas-
Prescribed fire	Induce seed germination and seedling elimination	Destruction of other species seed banks/seedlings; Alteration of soil properties and increment of soil erosion; Possibility of spreading to non-target areas; Socially controversial.	Drastic reduction of initial and follow up control costs; Very high seed germination and mortality; Reduce fuel loads; Control the season and local of fire occurrence.
Post Wildfire intervention	Seedling elimination	Unpredictability;	Drastic reduction of

Method	Objective	Disadvantages	Advantages
		Immediate action is needed after occurrence (few months); Instant availability of funds; Profound knowledge of species location.	initial control costs; Prevention of re-invasion in recently burned areas.
Biological control	Reduce seed numbers/ constraining seed production/ constraining plant development	Socially controversial; Risk that the control agent interacts negatively with native organisms; Cost consuming safety tests.	Once it is established costs are very low; Does not causes any disturbances; Can be implemented in inaccessible areas; No need of follow up control actions. Species specific

2.5 Conclusion

Acacia dealbata accumulates a large soil seed bank that is mostly viable. Most seeds remain dormant in soil, but 60ºC are enough to stimulate a considerable part of the seed bank. Although seeds are concentrated under the canopy, some seeds were actually found in adjacent areas, outside the invaded stands, stressing the need to consider these areas in management plans. The seed bank functions as a hidden legacy that can remain in soil for a long time, implying that the simple removal of *A. dealbata* individuals is a temporary solution. Our results highlight that the seed bank of species accumulating a numerous and long-lived seed bank needs to be seen as a significant part of the invasive ability of the species, and must be, therefore, incorporated in management programs.

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3. Final considerations

The tree *Acacia dealbata* is one of the most aggressive invasive species in Portugal, constituting a great conservation problem and a threat to native ecosystems (Marchante *et al*, 2008a). As demonstrated in this work this species has an extensive seed bank which is well beyond the invaded stands, functioning as a hidden legacy that can last for over 50 years (Richardson & Kluge, 2008; Gibson *et al*., 2011; Gioria *et al.*, 2012; Wijayabandara *et al.*, 2013). As found, this seed bank includes mostly dormant seeds but has the ability to germinate as soon as a disturbance or heat shocks occurs. Moreover, as it was demonstrated an extreme soil temperature caused by a heat wave has the ability to break seed dormancy, without the need of further disturbances, promoting species invasibility.

Consequently, removing standing plants is not enough to succeed in *Acacia dealbata* control because massive seed germination after clearing can jeopardize initial control efforts (Fernandes, 2008). Therefore seed bank management needs to be included in control programs.

There are several possible methods that can be used in order to deplete soil seed bank, but none is completely effective on its own. Taking advantage of the occurrence of wildfires can be part of the solution but the use of biological control agents such as Coletoptera *Melanterius maculates* should be considered, as this can be a fundamental tool to control this invasive species.

Controlling *Acacia dealbata* is, and will be, an extremely difficult task. Thus, to achieve this goal it is crucial to include seed bank management in control programs, along with individual's removal and follow up actions.

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