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Evaluation of the recovery of areas invaded by silver wattle in the Paisagem protegida da Serra do Açor

Dissertação no âmbito do mestrado em Ecologia orientada pela Professora Doutora Helena Maria de Oliveira Freitas e pela Doutora Elizabete Maria Duarte Canas Marchante e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

Outubro de 2021

Agradecimentos

Como não fiz esta caminhada sozinha, queria agradecer a todos os que me acompanharam, nomeadamente:

À Dra. Elizabete Marchante, por ter aceitado ser minha orientadora, por todo o encorajamento que me deu, por toda a paciência que teve comigo e por todo o material disponibilizado;

Ao Instituto de Conservação da Natureza e das Florestas, pela oportunidade de fazer este estudo na Paisagem Protegida da Serra do Açor; ao Nuno, ao Luís, ao Horácio, à Maria, à Fátima, à Ana, à Susana, ao Hugo, ao Daniel e ao Ricardo, por me terem acolhido e me terem acompanhado no trabalho de campo;

À Dra. Sílvia Neves, por se ter disponibilizado a me ajudar a dar os primeiros passos no trabalho de campo;

À Dra. Helena Freitas, por ter aceitado ser minha orientadora;

À minha família, por todo o apoio que me têm dado, sem a vossa ajuda não teria chegado até aqui;

A todos os amigos que estiveram comigo durante a licenciatura e o mestrado, gosto muito de vocês!

This work was carried out at the R&D Unit Centre for Functional Ecology - Science for People and the Planet (CFE), with reference UIDB/04004/2020, financed by FCT/MCTES through national funds (PIDDAC).

Resumo

As plantas invasoras causam frequentemente impactes negativos nos ecossistemas, na economia e até na saúde pública, pelo que muitas vezes são necessárias medidas de controlo e de recuperação das áreas invadidas. A mimosa (*Acacia dealbata*) é uma das espécies de plantas invasoras com maior distribuição em Portugal continental. A produção de numerosas sementes, que se acumulam no solo durantes muitos anos e cuja germinação é facilitada pelo fogo, e o facto de rebentar vigorosamente de touça e/ou raiz após o corte ou fogo, são características que favorecem a invasão e contribuem para a rápida (re)invasão após incêndios ou ações de controlo. Na Paisagem Protegida da Serra do Açor (PPSA), o controlo desta espécie iniciou-se em 2004/2005, tendo sido efetuado o controlo inicial e vários controlos de continuidade ao longo dos anos, pelo que em muitas das áreas de intervenção a presença de mimosa era já residual em 2017. No entanto, uma grande parte da PPSA ardeu num incêndio em outubro de 2017 e, consequentemente, as sementes de mimosa acumuladas no banco de sementes, no solo, foram estimuladas e germinaram. Neste contexto, os objetivos deste estudo foram avaliar: 1) a recuperação da vegetação nativa nas áreas onde a mimosa foi controlada desde 2004/2005; 2) o sucesso das intervenções de controlo de mimosa; e 3) o banco de sementes de mimosa que permanece no solo. Ainda que de forma indireta, foi tida em consideração a contribuição da reinvasão por mimosa depois do incêndio de 2017 e de subsequentes ações de controlo para a recuperação das áreas previamente invadidas. Para tal, na PPSA, analisaram-se vários parâmetros em áreas sujeitas a números diferentes de controlos de continuidade, que arderam no incêndio de 2017: a riqueza específica e cobertura de espécies nativas; a cobertura, número de exemplares e altura de mimosa; e o banco de sementes de mimosa acumulado no solo. Em termos de recuperação de espécies nativas, observou-se um total de 54 espécies distribuídas pelas áreas amostradas, refletindo-se em coberturas superiores a 60%, e não se observaram diferenças significativas na riqueza específica, independentemente do número de controlos de continuidade (média entre 4.9 e 9,4 espécies/área). Independentemente do número de controlos de continuidade e depois do incêndio, de forma geral, a cobertura de mimosa observou-se abaixo dos 10% e o número de espécimes foi inferior a 10, sendo estes de pequena dimensão (menores de 115 cm). Encontraram-se poucas sementes de mimosa no solo (23 no total das áreas), o que revela que após os vários controlos de continuidade (que na maioria dos casos impediram a formação de novas sementes de mimosa) e após o incêndio de 2017 (que estimulou a germinação das sementes acumuladas maioritariamente antes das intervenções de controlo iniciarem em 2004) já não existem muitas sementes acumuladas no solo. Estes resultados realçam que os controlos de continuidade são cruciais para, por um lado, diminuir a cobertura de mimosa e consequentemente tornar a recuperação da vegetação nativa possível; e, por outro lado, para assegurar que o banco de sementes não é reabastecido ao longo dos anos. No entanto, as intervenções de controlo da mimosa foram feitas apenas a nível das plantas, não eliminando as sementes que permanecem viáveis no solo por muitos anos. Como se observou na PPSA após o incêndio de 2017, isto refletiu-se na reinvasão por mimosa após o incêndio, alertando para a necessidade de estar alerta e assegurar o rápido controlo das plantas que germinarem.

Apesar de estes resultados serem limitados a uma área de estudo, têm implicações importantes em termos de gestão de plantas invasoras. Por um lado, realçam a importância crucial de assegurar os controlos de continuidade a médio/longo prazo, em especial quando se trata de plantas invasoras com capacidade para regenerar de raiz e/ou touça após o corte e que acumulam banco de sementes duradouros. Por outro lado, alertam para o potencial de reinvasão “escondido” no banco de sementes do solo, que não pode ser descurado, em especial após incêndios ou outros eventos que estimulem a germinação das sementes.

Palavras-chave

Acacia dealbata, fogo, controlo de plantas invasoras, recuperação ecológica, invasão por espécies exóticas

Abstract

Invasive plants frequently promote negative impacts in ecosystems, in economy and even in public health; as such, control measures along with recovery of invaded areas are often necessary. Silver wattle (*Acacia dealbata*) is one of the invasive plant species with largest distribution in mainland Portugal. Production of numerous seeds, which accumulate in the soil for many years and whose germination is facilitated by fire, and resprouting vigorously from stumps and/or roots after cuts or fire, are characteristics that favour invasion and contribute to the fast (re)invasion after fires or control actions. In Paisagem Protegida da Serra do Açor (PPSA), the control of this species started in 2004/2005, having been carried out the initial control and several follow-up controls over the years, so in many of the areas of intervention the presence of silver wattle was residual in 2017. However, a big part of PPSA burned in a fire in October of 2017, and consequently the seeds of silver wattle that were accumulated in the seed bank, in the soil, were stimulated. In this context, the aims of this study were to evaluate: 1) the recovery of native vegetation in areas where silver wattle was controlled since 2004/2005; 2) the success of silver wattle controls; and 3) the silver wattle seed bank that remains in the soil. Though indirectly, it was taken into consideration the contribution of reinvasion by silver wattle after the fire of 2017 and subsequent control actions to the recovery of previously invaded areas. For such, in PPSA, several parameters in areas subject to different numbers of follow-up controls and that burned in the fire of 2017 were analysed: richness and cover of native species; cover, number of specimens and height of silver wattle; and silver wattle seed bank accumulated in the soil. In terms of recovery of native species, a total of 54 species was observed, distributed throughout the sampled areas; this was reflected in plant cover above 60%, and no significant differences in species richness, regardless of the number of follow-up controls (average between 4.9 and 9.4 species/area). Independently of the number of follow-up controls and after the fire, in general, silver wattle cover was below 10% and the number of specimens was less than 10, these being small in size (smaller than 115 cm). Very few silver wattle seeds were found in the soil (23 in all the areas), which reveals that after the several controls (which in most cases prevented the formation of new silver wattle seeds) and after the fire of 2017 (which stimulated the germination of the seeds accumulated mostly before the control interventions started in 2004) there aren't many seeds accumulated in the soil anymore. These results highlight that follow-up controls are crucial to, on the one hand, reduce the cover of silver wattle and

consequently make the recovery of the native vegetation possible; on the other hand, to ensure that the seed bank isn't restored over the years. However, the interventions of control of silver wattle were made only at plant level, not eliminating the seeds that remain viable in the soil for many years. As observed in PPSA after the 2017 fire, this resulted in the reinvasion of silver wattle after the fire, alerting for the need to be alert and to ensure the fast control of plants that germinate.

Although these results are from only one study area, they have important implications in terms of management of invasive plants. On the one hand, they highlight the crucial importance of assuring the follow-up controls at medium/long-term, especially when concerning invasive plants with the ability to regenerate from the root and/or stump after cutting and which accumulate long-lasting seed banks. On the other hand, they alert for the potential of reinvasion "hidden" in the soil seed bank, which cannot be overlooked, especially after fires or other events that stimulate the germination of seeds.

Keywords

Acacia dealbata, fire, control of invasive species, ecological recovery, invasion by exotic species

Índex-

Introduction

A biological invasion happens when an alien species (species introduced outside its native range) spreads and establishes self-sustaining populations into areas far away from sites of introduction without human intervention (Richardson *et al.*, 2000); this may be reflected in high densities of the species that may promote impacts at different levels. More pronouncedly in the last 200 years, people have introduced, on purpose or accidentally, thousands of alien species around the world and continue introducing them with no sign of stopping (Seebens *et al*., 2017). Since many alien species become invasive, there are numerous invasive alien species (IAS) spread all around the globe in many different ecosystems, reducing biodiversity worldwide. In fact, IAS have been considered globally as the fifth biggest threat to biodiversity (IPBES, 2019). Previously, the United Nations (UN) had already included in their Sustainable Development Goals regarding Life on Land - SDG 15 (United Nations, 2019) a specific target about IAS, recognizing the huge threat biological invasions represent for global sustainability: Target 15.8 aimed, by 2020, to introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species on land and water ecosystems, and control or eradicate the priority species. Today, many countries have legislation to prevent the introduction of alien species, but this target was far from been achieved in 2020 and is presently being restructured for 2030.

The impacts of IAS are very context-dependent, varying from positive to negative, and depend also on the stakeholders involved and different perceptions (Kull *et al.*, 2011; Shackleton *et al.*, 2019a; Shackleton *et al.*, 2019b). Some invasive species of animals and plants can be useful to people, for example, as part of their diet, but simultaneously they can be a threat to native species and ecosystems (Simberloff *et al.*, 2013). Considering invasive alien plants in particular, they often cause impacts at multiple levels such as at individual, population, community and ecosystem level (Ricciardi *et al.*, 2013). Common impacts are the decrease of abundance and diversity of native species, alterations of nutrient and mineral content in native plant tissues, modifications of soil components and changes in fire frequency and severity (Marchante *et al*., 2008; Le Maitre *et al.*, 2011; Vilà *et al*., 2011; Pyšek *et al.*, 2012; Marchante *et al*., 2015). In many invaded areas, communities become simpler when compared to non-invaded areas as the richness and abundance of both plant and animal species decline (López-Núñez *et al.*, 2017). Additionally, entire ecosystems can be transformed since many invasive alien plants can alter ecological networks, habitat structure and soil ecology and functioning (Marchante *et al*., 2008; López-Núñez *et al.*, 2017; Pyšek *et al.*, 2020). When it comes to the impacts on people more directly, invasive alien plants can have positive impacts, for example when populations gain new resources in a specific area, sometimes vital for the survival of local populations, but they can also have negative impacts, for example when as a result of an invasive plant populations have reduced agricultural production and even health issues (Shackleton, Shackleton and Kull, 2019).

Considering the significant and extensive negative impacts of IAS, managing and controlling them is often necessary, especially in Protected Areas or when they impact negatively human activities. However, controlling IAS is a complex and hard task, especially when the invasive species are not detected early, since the resources required to manage them, not only financially, but also in terms of time, technical and human resources are very demanding (Le Maitre *et al.*, 2011; Simberloff *et al.*, 2013).

Portugal is no exception when it comes to biological invasions. As in other Mediterranean areas, the number of IAS and the impacts they promote are substantial and often negative, as was recently reviewed by Vicente and collaborators (2018). The government has been making an effort to tackle the threat of invasive species namely by regulating the introduction and management of alien species (Decreto-Lei nº 92/2019, Ministério do Ambiente, 2019). This decree-law, revises the previous regime established by Decree-Law No. 565/99 (Ministério do Ambiente, 1999), and implements one of the measures provided in the National Strategy for Conservation of Nature and Biodiversity 2030 (Presidência do Conselho de Ministros, 2018). Simultaneously, it gives full execution at national level to the regime established by the European Union Regulation No. 1143/2014, of the European Parliament and of the Council, of October 22, 2014, concerning the prevention and management of the introduction and propagation of invasive alien species. The present national legislation includes the National List of Invasive Species where more than 300 invasive plant species and over 100 invasive animal species are listed.

Among the aquatic invasive plants, one of the most widespread and with more negative impacts in Portugal and in the world is water hyacinth - *Eichhornia crassipes* (Mart.) Solms*;* this aquatic plant grows extremely fast and creates long thick mats, which often causes various negative impacts at ecological and socio-economical levels in the rivers and lakes it invades (Villamagna and Murphy, 2010; Marchante *et al*., 2014; Marchante and Marchante, 2020). Another example of an invasive alien plant in

Portugal is the ice plant - *Carpobrotus edulis* (L.) N. E. Br. - a succulent that invades mainly dune ecosystems throughout the coastal areas, acidifies the soil and replaces the native vegetation to a large extent (Campelo, 2000; Conser and Connor, 2009; Marchante *et al*., 2014). Another widespread invasive alien plant is pampas grass - *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. - a perennial grass that invades dunes and urban areas, forms dense stands and creates barriers for local fauna (Domenech and Vilà, 2007; Marchante *et al*., 2014). In agricultural areas, a common invasive alien plants is Bermuda buttercup - *Oxalis pes-caprae* L. – a perennial herb that invades crop lands and wastelands, forms dense mats that reduce the development of native vegetation and lowers productivity in crop fields (Petsikos, Dalias and Troumbis, 2007; Marchante *et al*., 2014). As for terrestrial woody species, the genus *Acacia* is possibly the most widespread in Portugal mainland with significant impacts at diverse levels, from plant and gall communities to soil ecology (Marchante *et al*., 2008; Le Maitre *et al.*, 2011; Marchante *et al*., 2015; López-Núñez *et al.*, 2017). Nowadays, the all genus is considered invasive by the National legislation (Decreto-Lei nº 92/2019, Ministério do Ambiente, 2019).

Among the *Acacia* species invasive in Portugal, silver wattle (*Acacia dealbata* Link.) is one of the most widespread in the mainland. This is a tree of the *Fabaceae* family native to Australia, more specifically to New South Wales, Victoria and Tasmania. It can reach 15 m in height and it is easily recognizable by its evergreen and greyish-green leaves and its yellow flowers in globular flower heads; the flowering occurs in general between January and April; and the fruits are brown-reddish pods (Marchante *et al*., 2014) (Figure 1). Silver wattle is considered one of the most widespread and impactful invasive terrestrial plant species in Portugal, as it is present in most provinces of the territory (except Azores) (Figure 2) and it forms dense and extensive populations in many areas, including in Protected Areas (Lorenzo *et al*., 2010; Marchante *et al.*, 2014; ICNF, 2019). This species was introduced in Europe in the late 1700s (Sheppard *et al*., 2006) and was then used as an ornamental plant in the 19th century in southern Europe (Lorenzo *et al*., 2010). Silver wattle can disturb native plants through allelopathy, evident in individual factors like inhibition of photosynthesis and germination, but also population factors like species richness, plant density and cover, causing biodiversity loss (Lorenzo *et al*., 2011; Lorenzo *et al*., 2012). Being an N-fixing species, it can also modify soil characteristics and functioning (Le Maitre *et al.*, 2011; González-Muñoz, Costa-Tenorio and Espigares, 2012). This *Acacia* species, as *Acacia melanoxylon* R. Br., often invades along rivers and streams and as such it may change water quality (increase in N concentration) and quantity (decrease in flow) and alter litter input characteristics (altered diversity, seasonality, typology, quantity and quality) (Ferreira *et al.*, 2021). Another relevant characteristics of this species that further contributes for its invasive behaviour is the production of numerous seeds which accumulates on persistent seed banks whose germination is promoted by fire and disturbance (Richardson and Kluge, 2008; Passos *et al*., 2017; Gioria *et al*., 2019).

Figure 1. Silver wattle (*Acacia dealbata*) with evergreen greyish-green leaves, yellow flowers and brown pods. Source: Plantas Invasoras em Portugal (2021).

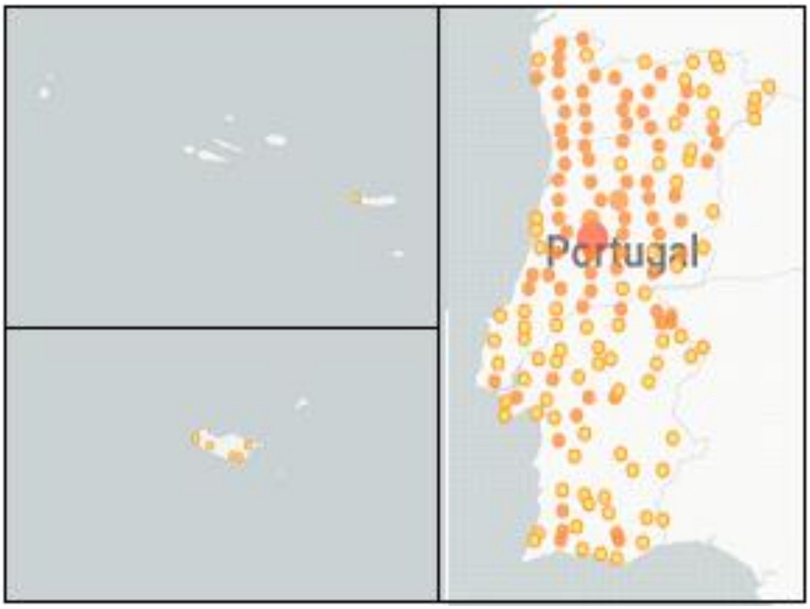


Figure 2. Presence of silver wattle (*Acacia dealbata*) in Portugal, retrieved from the Global Biodiversity Information Facility (GBIF)(2021). GBIF image include datasets: Sightings Map of Invasive Plants in Portugal, iNaturalist Research-grade Observations, Flora-On: occurance data of the flora of mainland Portugal, Pl@ntNet automatically identified occurrences, and Biodiversity4all Research-Grade Observations; bigger and darker dots means more species records.

Study site

Paisagem Protegida da Serra do Açor (PPSA) is a Protected Area in the municipality of Arganil, in the district of Coimbra, in the centre of Portugal (Figure 3). It has 382 ha and includes Mata da Margaraça with 50 ha (Figure 4), a relic of the primitive vegetation that deserves special conservation status (Decreto-Lei nº 67/82, Ministério da Qualidade de Vida, 1982). This Protected area has a temperate climate with dry summers (IPMA, 2021) and is part of the Rede Nacional de Áreas Protegidas (Portuguese National Network of Protected Areas) and (co)managed by Instituto da Conservação da Natureza e das Florestas (ICNF). Mata da Margaraça is a temperate deciduous forest with relict taxa (A Figueiredo personal communication).PPSA has 10 different units of vegetation, with the two most common being forests with deciduous trees and pine forests (ICNF, 2007). The dominant plant species are oaks (*Quercus robur* L.), chestnuts (*Castanea sativa* Mill.), Portuguese laurel (*Prunus lusitanica* L.) and laurel (*Laurus nobilis* L.) (ICNF, 2007).

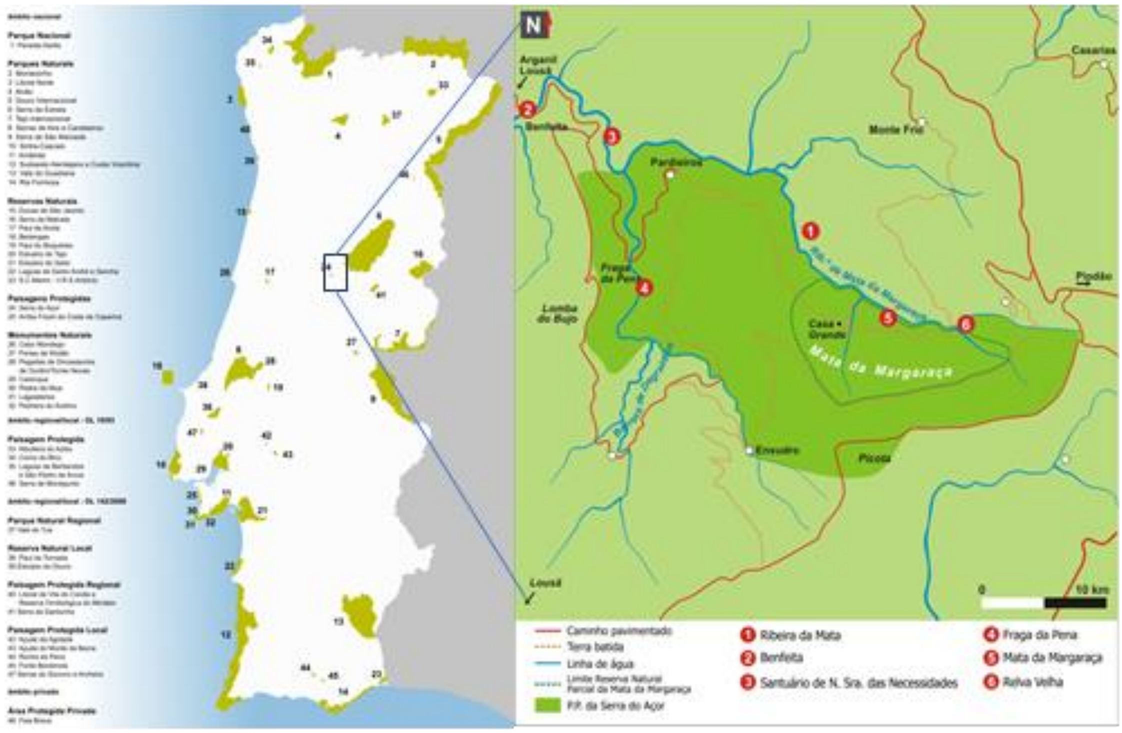


Figure 3. Location of the study site, Paisagem Protegida da Serra do Açor (PPSA), in the centre of Portugal. Source: ICNF, 3/11/2020

Figure 4. Mata da Margaraça (left); Paisagem Protegida da Serra do Açor (PPSA) (right). © Cristina Girão Vieira.

At PPSA, silver wattle invaded several (18) relatively small areas (most with less than 1 ha) around Mata da Margaraça. These areas were mostly agricultural areas, pine forests and forests with native species like oak and chestnut (ICNF, 2007). In 2004/2005, a control program was initiated, aiming to protect Mata da Margaraça from the invasion (ICNF 2013) (Figure 5). The method used for the initial control was cutting the trees and application of herbicide in the stumps (Figure 6), but this was expected to result in reinvasion from resprounting of stumps and/or roots and germination of seeds, and as such follow-up controls were planned from the beginning of the control program. The follow-up controls were made annually, or every two years, by hand pulling the resprouts and new plants when possible, or cutting them and applying again herbicide in the small stumps (ICNF, 2013; Annex I; Figure 7). By March 2020, all areas had between eight and 12 follow-up controls (Table 1).

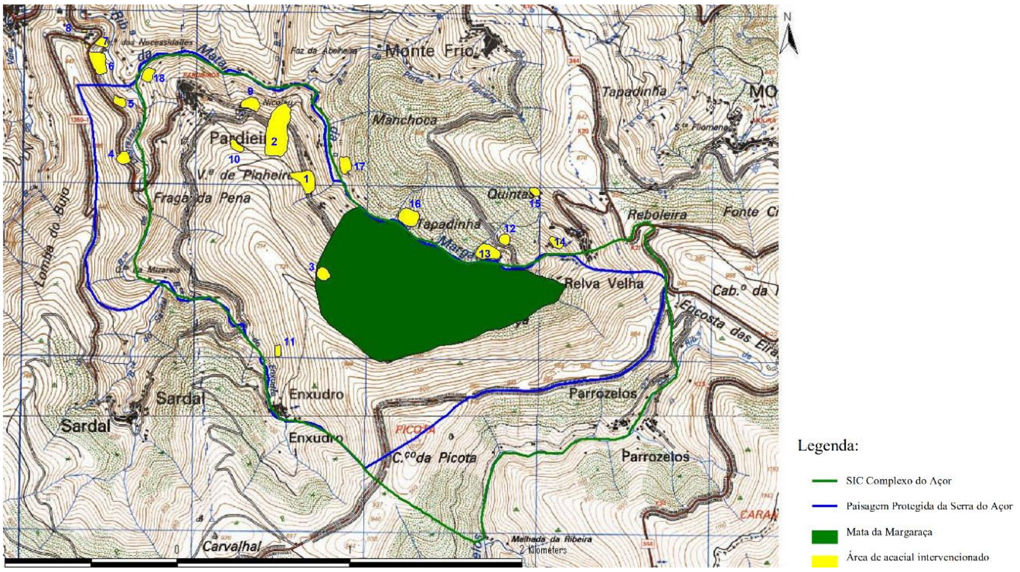


Figure 5. Areas initially invaded by silver wattle and subject to control interventions since 2004/2005 by ICNF (yellow areas with numbers from 1 to 18). The green area is Mata da Margaraça and the blue line is the limits of Paisagem Protegida da Serra do Açor (PPSA).

Table 1. Number of controls of each invaded area by Instituto da Conservação da Natureza e das Florestas (ICNF). Areas are numbered from 1 to 18 (as in Figure 5).

Number of follow-up controls (by March 2020)	Area codes
8	2, 10, 17, 18
9	9, 11, 13, 14, 15, 16
10	3, 12
11	4, 5, 6, 7, 8
12	1

Figure 6. Initial control of silver wattle by Instituto da Conservação da Natureza e das Florestas (ICNF) in 2004 and 2005 with cutting the trees (left) and applying herbicide in the stumps (right). © Sílvia Neves.

Figure 7. Follow-up controls of silver wattle by Instituto da Conservação da Natureza e das Florestas (ICNF) after 2004 with hand pulling the resprouts and new plants. © Sílvia Neves.

With several follow-up controls along time, these control actions were quite effective, and by 2017 controlled areas didn't show many specimens of silver wattle (ICNF personal observation; Rodrigues, 2014), which is corroborated by the continuous decrease on the number of hours needed to control the areas as the number of follow-up controls increased (Figure 8, Annex I). However, the seeds still remained stored and viable in the soil. According to Rodrigues (2014), in 2013 controlled areas still had silver wattle seeds in the soil, despite in the majority of controlled areas there was no seed production since the beginning of the control program back in 2004/2005. In October 2017, Portugal was affected by severe and extensive fires throughout the territory (Bladon, 2018) and a considerable part of PPSA burned (Figure 9). After the fire, areas where silver wattle had been controlled faced a likely reinvasion since seeds of the species were still stored in the soil seed bank and would be most probably be stimulated to germinate (Richardson and Kluge, 2008). In fact, this was observed a few months after the fire and ICNF has controlled such germination in most areas; this is corroborated by the increase in the number of hours of work spent controlling silver wattle right after the 2017 fire (Figure 8, Annex I).

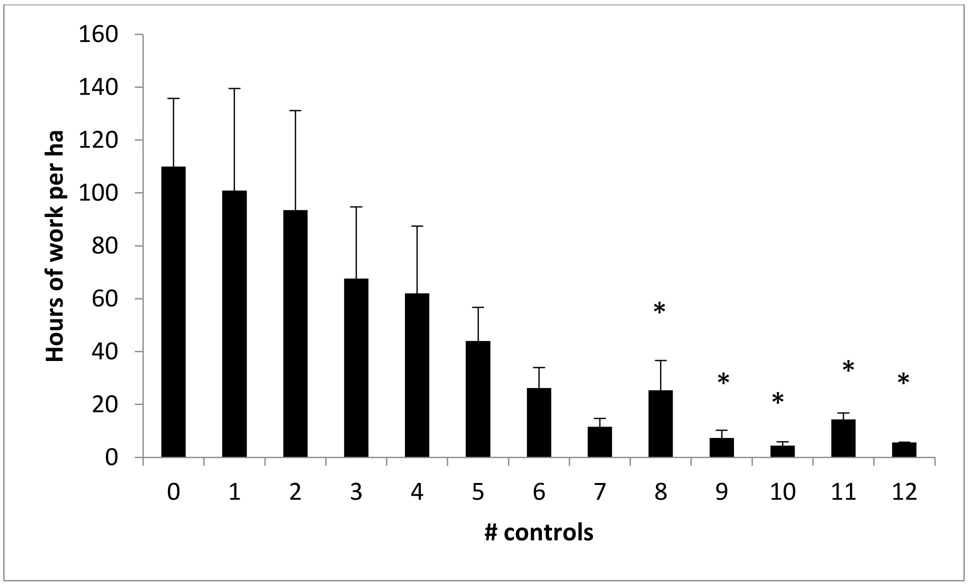


Figure 8. Number of hours (average number per hectare + SE) spent controlling silver wattle in invaded areas per number of follow-up control. Bars with * include controls from before and after the 2017 fire; other bars only had controls before the fire. The number of follow-up controls is different in the different areas. Data not published from ICNF, PPSA.

Figure 9. Paisagem Protegida da Serra do Açor (PPSA) after the fire in October 2017. © Elizabete Marchante.

Objectives

Considering the above-mentioned context, where silver wattle has been controlled since 2004/2005 in the Paisagem Protegida da Serra do Açor (PPSA), with follow-up controls being ensured since then, and the fact that a large area of PPSA burned in 2017, the main objectives of this study were to evaluate: 1) the recovery of native vegetation in areas where silver wattle was controlled since 2004/2005; 2) the success of silver wattle controls; and 3) the silver wattle seed bank that remains in the soil.

Based on the results of previous studies (ICNF, 2013; Rodrigues, 2014), that show the recovery of native vegetation and an increasing reduction in the presence of silver wattle as the number of follow-up controls increase, on the known characteristic of the species, namely its ability to accumulate long-lived seed banks and germinate after fire (Richardson and Kluge, 2008; Le Maitre *et al.*, 2011; Passos *et al*., 2017), and the fact that the area burned in 2017, the hypotheses for this study are: (1) areas where more follow-up controls were done will show increasing recovery of native vegetation and (2) lower presence of silver wattle (both cover and number of specimens), although this may be confused by the effect of the fire; and (3) the soil seed bank of silver wattle will be diminished after the 2017 fire since many of remaining seeds will have been stimulated to germinate after the fire.

Methods

Experimental design

Vegetation recovery

To evaluate silver wattle and native vegetation recovery in PPSA after control interventions and also after the 2017 fire, of the 18 areas subjected to silver wattle control 11 were selected (Figure 10), including areas with different number of follow-up controls: i) two areas with eight follow-up control interventions, ii) four areas with nine, iii) two areas with 10, iv) two areas with 11 and iv) one area with 12 follow-up control interventions[1]. Additionally, three non-invaded areas, without silver wattle, in the surroundings of the areas subjected to control were selected for comparison. A total of 14 areas were selected and sampled.

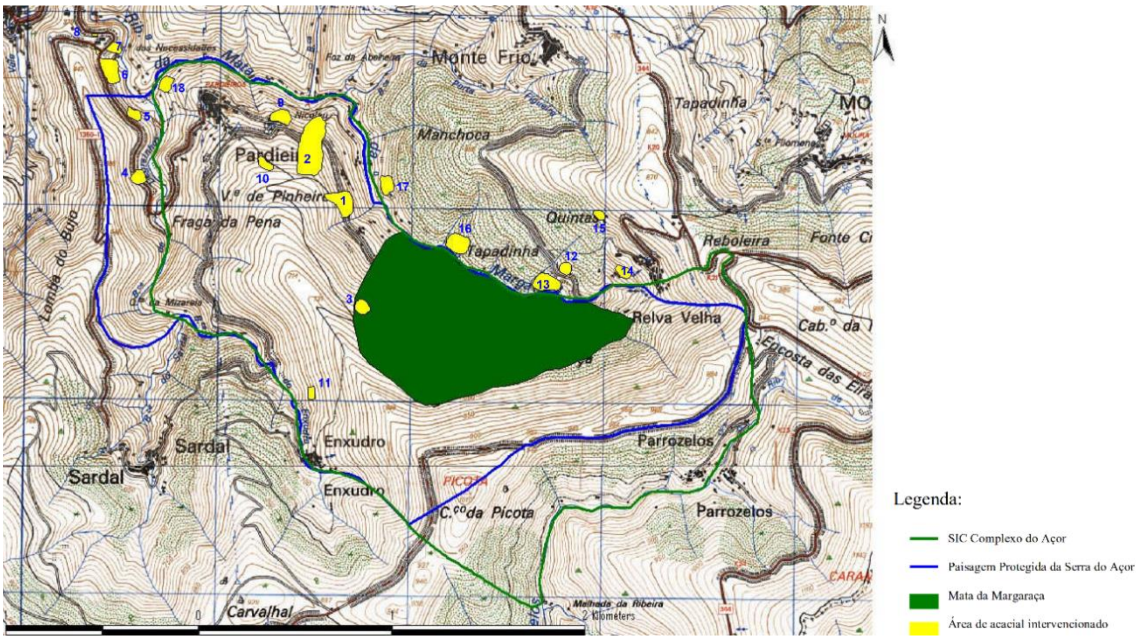


Figure 10. Areas initially invaded by silver wattle and subject to control interventions since 2004/2005 by ICNF (yellow areas). The areas selected for this study are 1, 2, 3, 4, 5, 10, 11, 12, 14, 15 and 16. The green area is Mata da Margaraça and the blue line is the limits of Paisagem Protegida da Serra do Açor (PPSA).

[1] Initially, three areas were selected per number of follow-up controls, but before the sampling, ICNF workers did another control intervention in some of the areas, resulting in an uneven number of replicates per number of follow-up controls.

In each of the selected areas, five plots of 4 m^2 (2 x 2 m) were sampled in May 2020, totalizing 70 plots (Figure 11, Figure 12). The size of the plots was defined considering that most of the vegetation was herbaceous and shrubby and also in order to compare the results with previous work developed in the same study area (Rodrigues 2014). In each plot, all plant species present were registered, as well as their cover percentage. When species could not be identified in the field, plants were collected for further identification in the laboratory using Nova Flora de Portugal (Franco, 1971, 1984; Franco and Afonso 1994, 1998, 2003); when the phenological stage at time of sampling did not allow species identification, the taxa was identified to family or genus level. To evaluate silver wattle recovery, several parameters were evaluated: i) plant cover, ii) number of specimens, and iii) height of the specimens.

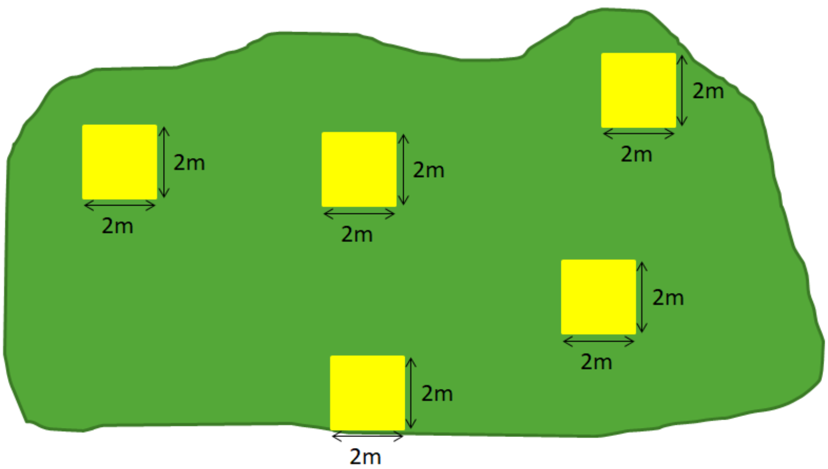


Figure 11. Experimental design to evaluate vegetation recovery. In each area (green zone), five plots of 4 m^2 (2 x 2 m) were delimited. Each plot (yellow squares) was randomly assigned and had a distance of at least 3 m from the closest one.

Figure 12. Field work registering the different parameters evaluated in the sampling plots.

Seed bank of silver wattle

Since the seed bank of silver wattle is numerous, long-lived and is stimulated by fire, it is very relevant to evaluate it when evaluating the recovery of areas invaded by this species, particularly since a large proportion of PPSA has burned in 2017. Therefore, in order to evaluate the soil seed bank of silver wattle, from November 2019 to February 2020, three types of burned areas were selected and sampled: i) three areas with eight follow-up control actions, ii) three areas with 11 follow-up control actions, and iii) three areas with native vegetation (that has never been invaded by silver wattle). These areas were selected in order to sample areas with the most distinct number of follow-up controls[2], and, consequently, with greater chance of differences in the seed bank to be detected. In each of the nine areas, three transects of 30 m were marked and soil samples collected every 3 m (11 samples per transect), summing 33 samples per area and 297 in total (Figure 13, Figure 14). A metal core of 10 cm of height and 8 cm of diameter was used to collect the soils samples, which were properly identified and transported in plastic bags. Each sample included two sub-samples, one on each side of the transect, in order to increase the area of sampling and representativeness. Samples were deposited on trays to dry at room temperature, and when dried, they were carefully screened in order to select and count silver wattle seeds. To check if seeds were viable they were scarified and placed in plates for 30 days or until they germinated or rotted.

[2] When the work was planned the maximum number of treatment was 11, not 12 as latter happened.

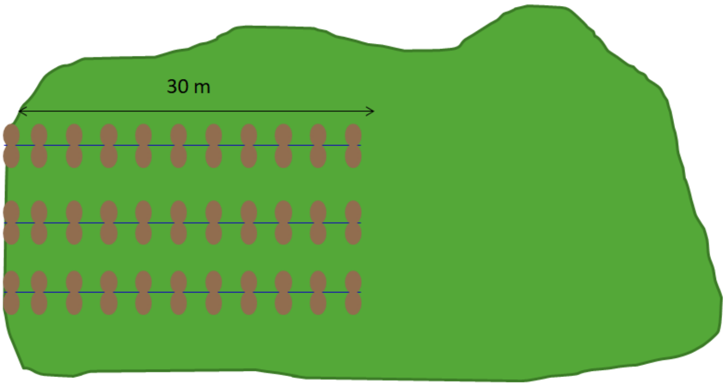


Figure 13. Experimental design to evaluate the silver wattle soil seed bank. In each area (green zone), three transects of 30 m were demarked. Each transect (blue lines and brown circles) started in the limits of the area when possible (0 m) and distanced at least 3 m from the other closest transect. Every 3 m, two samples of soil were collected (sets of two brown circles) using a metal core with 10 cm of height and 8 cm of diameter.

Figure 14. Field work collecting samples to evaluate the silver wattle soil seed bank.

Statistical analysis

Species were distributed by family and richness of native species was used to evaluate the proportion of plant families recovering in areas with different numbers of follow-up controls of silver wattle.

For each of the parameters analysed, i.e., plant richness, plant cover, and silver wattle cover, number of specimens, height of specimens, and number of seeds in the soil seed bank, a one factor analysis of variance (ANOVA) was performed to determine if there were significant differences between the different areas evaluated, considering the number of follow-up controls as factor. Before performing the ANOVA, the Shapiro-Wilk test and the Levene test were performed to check the assumptions of ANOVA. When these were fulfilled, the ANOVA was performed. When they weren't fulfilled, the non-parametric Kruskal-Wallis test was performed. When significant differences were detected by the ANOVA or the Kruskal-Wallis test ($p<0.05$), a post-hoc test was performed. In the case of ANOVA, the post-hoc test was the Tukey HSD test; when the Kruskal-Wllis test was performed, an analysis of multiple comparisons of p values was used. The statistical analyses were performed using Statistica 7.

Results

Plant recovery

Overall, 54 plant species (Annex II) were identified in the study areas at Paisagem Protegida da Serra do Açor, i.e., in areas that had been subject to control of silver wattle since 2004/2005 and burned in October 2017. The species observed belong to 23 families; two unidentified species were not taken into consideration for this analysis since it was not possible to determine the family.

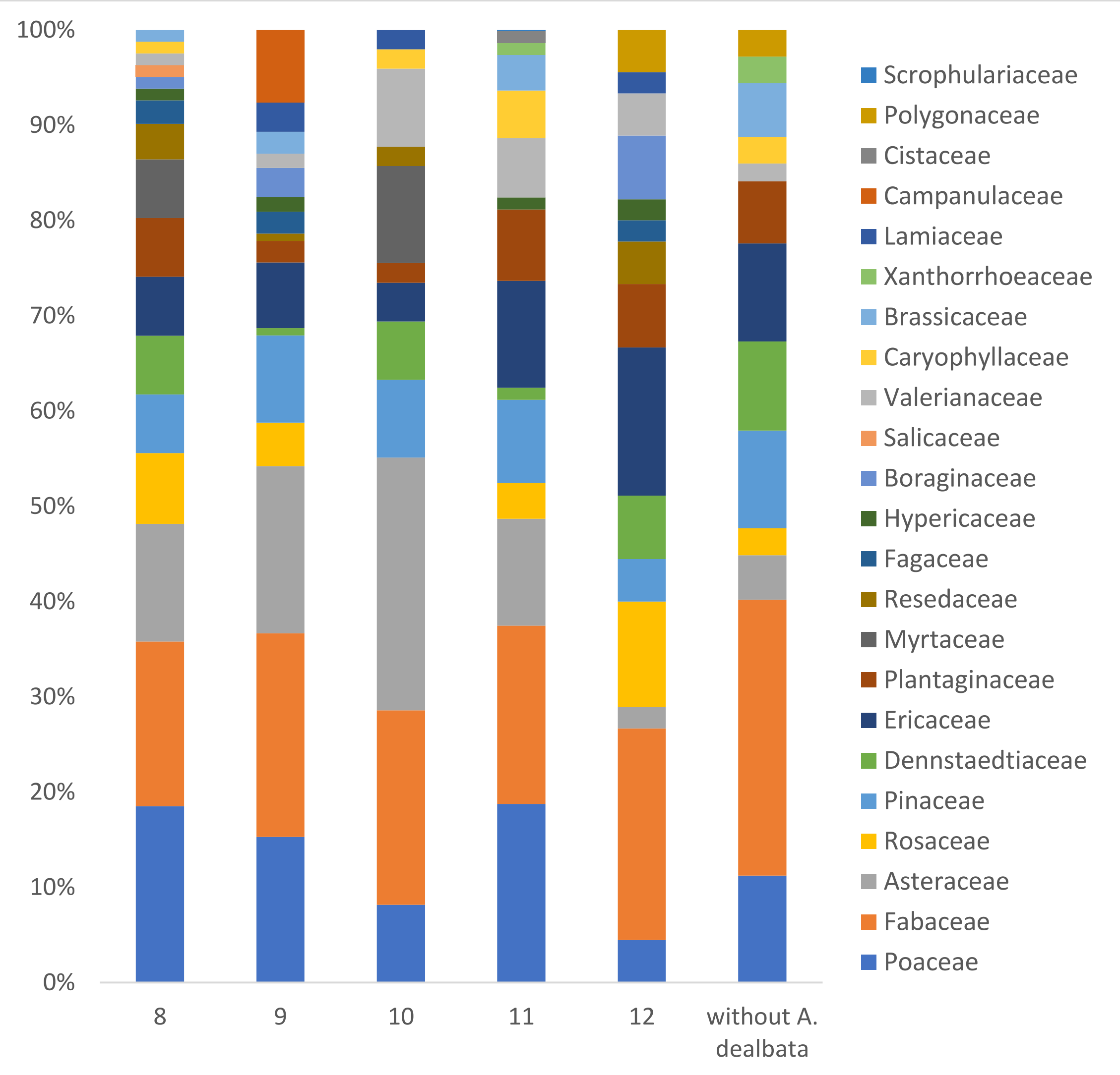
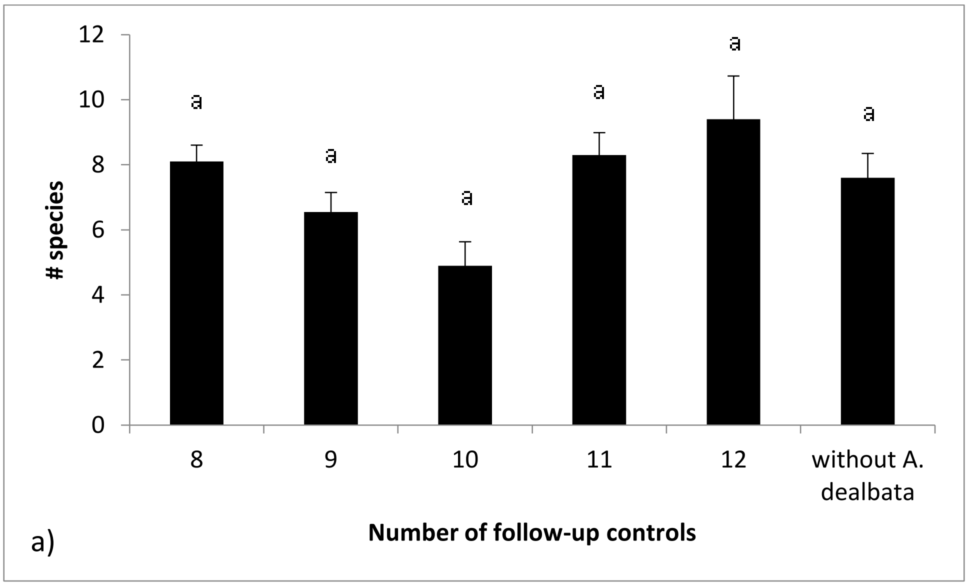


Figure 15. Plant families (proportion per type of treatment) present in areas subjected to different numbers of follow-up controls of *Acacia dealbata* and in non-invaded areas, in Paisagem Protegida da Serra do Açor. These results do not include *Acacia dealbata*, considering families of all other species present in the different plots and replicate areas.

The three most common plant families were Fabaceae, Poaceae and Asteraceae, but many other families were represented, e.g., Rosaceae, Plantaginaceae, Dennstaedtiaceae, Ericaceae and Pinaceae. The number of families doesn't appear to vary much depending on the number of follow-up controls, varying between 13 and 18 (Figure 15), although its apparent some tendency for Fabaceae and Ericaceae to increase and Poaceae and Asteraceae to decrease as the follow-up controls increase and ultimately in the non-invaded areas, possibly reflecting a tendency to the increase of shrubs and more perennial species.

The average number of species per type of area was low, varying between 4,9 and 9,4 (Figure 16a), but was quite variable between replicate plots: minimum two, in plots with 10 follow-up controls, and maximum 14, in plots with 12 follow-up controls. Consequently, the statistical analysis showed no differences in species richness of the different treatments (H=5, p=0.128; Annex III), independently of the presence of silver wattle and the number of follow-up controls. As for the plant cover (Figure 16b), it varied between ca. 60% and 100% (sometimes exceeding this, when several layers of vegetation overlaps), and it was statistically different (H=5, p=0.0025, Annex III) in some areas: areas with 10 and 11 follow-up controls had lower species cover than other areas.



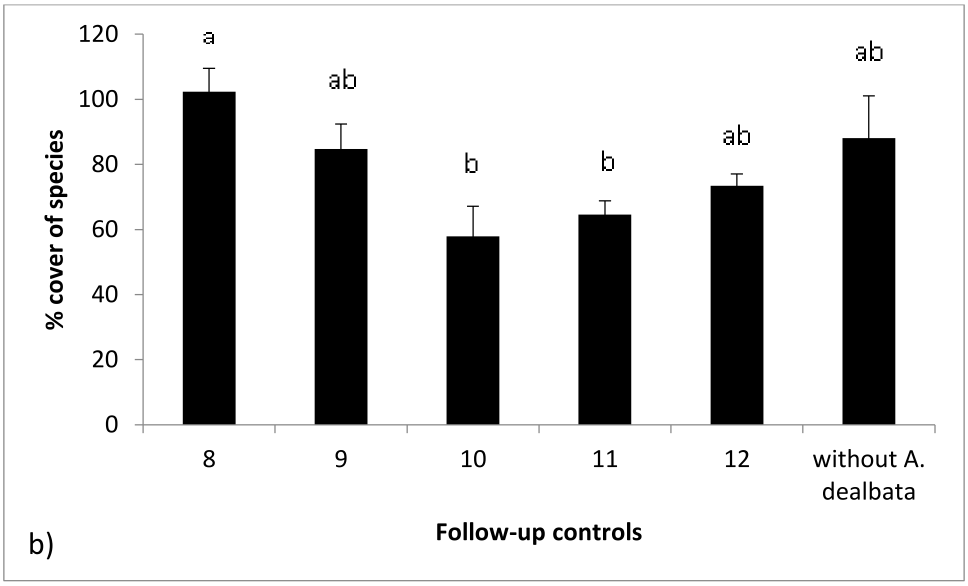


Figure 16. Species richness (average number + SE) (a) and cover of species (average + SE) (b) in areas subjected to different numbers of follow-up controls of *Acacia dealbata* and in non-invaded areas, in Paisagem Protegida da Serra do Açor. These numbers exclude *Acacia dealbata*. Same letters above bars mean that there are no significant differences between treatments (Kruskal-Wallis, $p<0.05$).

Silver wattle recovery

As for the recovery of silver wattle after the different follow-up controls, areas where controls occurred still show the presence of silver wattle, unlike non-invaded areas (without *A. dealbata* in Figure 17). Nevertheless, the cover of this species was in average below 10%, being even lower in most areas, showing that follow-up controls have been effective in reducing the invasion by silver wattle (Figure 17a). This is further corroborated by the low number of specimens of this species observed in the different sampled areas, which are in average below 10 specimens (Figure 17b), and that seldom reach 100 cm height (Figure 17c). For most of these parameters, the main significant differences were observed between areas that had been invaded and non-invaded areas, where silver wattle remains absent.

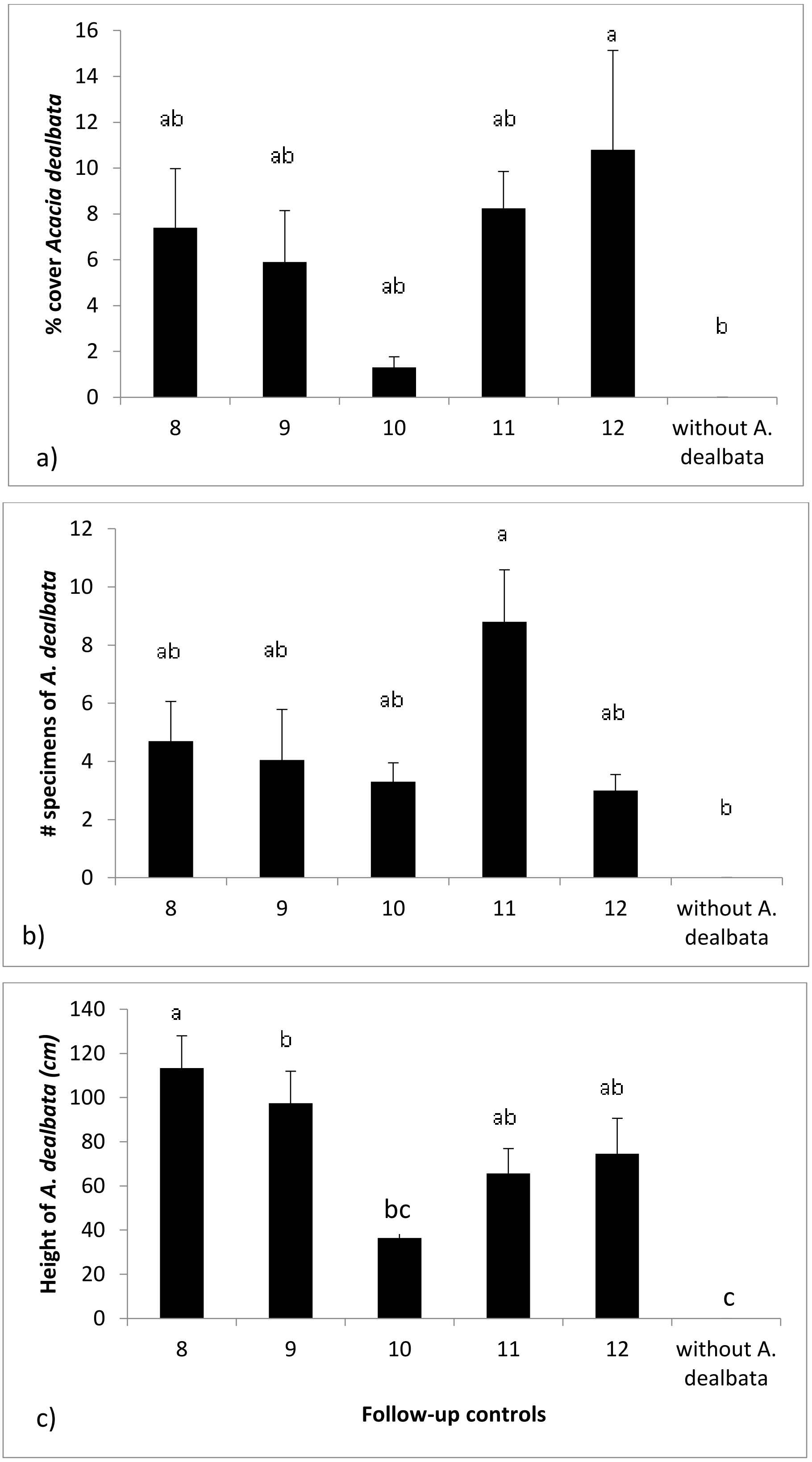


Figure 17. Cover (a), number (b) and height (c) of specimens of *Acacia dealbata* (average + SE), according to the number of follow-up controls of *A. dealbata* and in non-invaded areas, in Paisagem Protegida da Serra do Açor. Same letters above bars mean that there are no significant differences between treatments (Tukey HSD, $p<0.05$).

Silver wattle seed bank

When the soil seed bank was analysed, no seeds of silver wattle were found in areas with native vegetation (without *A. dealbata*) or in areas with 11 follow-up controls. On the contrary, a few seeds were found in areas with eight follow-up controls, 23 in total, corresponding to 0.23/cm^2 or 0.000023/m^2. However, the number of seeds was quite variable between replicates and the statistical analysis showed no differences between areas ($F=2.85$, $p=0.059$, Annex III) (Figure 18). Most of the seeds found were viable (87%, 20).

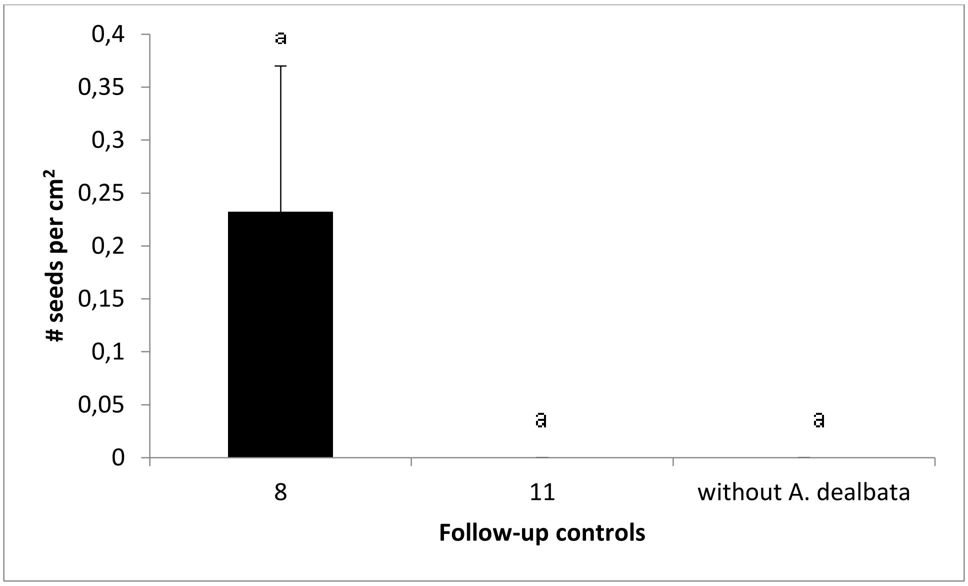


Figure 18. Number of *Acacia dealbata* seeds per cm^2 (average + SE) in areas subjected to different numbers of follow-up controls of *Acacia dealbata* and in non-invaded areas, in Paisagem Protegida da Serra do Açor. Same letters above bars mean that there are no significant differences between treatments (Tukey HSD, $p<0.05$).

Discussion

As mentioned in the introduction, silver wattle is one of the most widespread invasive plant species in mainland Portugal, but, despite many programs aiming to manage and control it along the country, results are often not enough successful and re-invasion occur in many cases (E Marchante, personal communication). This may be due to multiple factors, namely the characteristics of the species (i.e., its ability to resprout after cut by stumps and roots, and the production of numerous long-lived seeds that accumulate in persistent soil seed banks (Lorenzo *et al*., 2010; Gibson *et al*., 2011; Passos *et al*., 2017), the frequent absence or insufficient number of follow-up controls, the lack of long-term strategies, the inefficient application of control techniques, etc. (Lorenzo *et al*., 2010; Wilson *et al*., 2011). In this context, an initial single control is not enough to control the invasive silver wattle and follow-up controls are essential to achieve better results, since cutting stimulates resprouting and does not eliminate the seed bank (Lorenzo *et al*., 2010).

In PPSA, ICNF started controlling silver wattle in 2004/2005 (ICNF, 2013) and, even though the species is still present after more than 15 years, the reduction in terms of number and size of silver wattle specimens and percentage cover is evident: the present study clearly shows that although silver wattle is still present in areas subject to follow-up controls (ranging from eight to 12 follow-up controls by 2020), its presence is in low numbers (only a few individuals per plot, mostly less than nine, corresponding to plant covers of less than 10%, but much below this number in most sampled plots) and the specimens were quite small (the majority below 115 cm); the small size of the specimens denotes that they have not been producing new seeds. In 2013, when a previous monitoring was done, areas with three follow-up controls had still more specimens and higher cover of silver wattle (40 specimens per plot in areas with three follow-up controls and about 40% of cover), while areas with six follow-up controls showed results more close to the ones observed in this study, in 2020 (less than 10 plants per plot and less than 10% of cover; Rodrigues, 2014). In 2020, with few exceptions, all sampled areas, ranging from areas with eight to 12 follow-up controls, showed similar results. Although it could be expected that areas with more follow-up controls would have less silver wattle cover, this was not observed possibly because the number of follow-up controls is already high and the presence of silver wattle quite low. Additionally, it needs to be kept in mind that the present results also reflect the effects of the 2017 fire that confounds the effects of the follow-up controls: even though silver wattle is fire-adapted, and as such fire may have influenced recovery differently depending on the pre-fire abundance of the species, the fire burned most of the vegetation and confounded the final result.

As for the recovery of the native vegetation, the native vegetation cover ranging from 60% to 100% and the relatively similar plant richness in the different areas (including the non-invaded areas) observed in this study denotes that control interventions have been effective and vegetation is recovering. However, these numbers do not reflect the identity of the species present. The vast majority of species found were herbs like *Digitalis purpurea* L. and *Pteridium aquilinum* (L.) Kuhn, or shrubs like *Cytisus striatus* (Hill) Rothm. and *Rubus ulmifolius* Schott. Arboreal species like *Pinus pinaster* Aiton and *Quercus robur* achieved high covers but specimens were still mostly saplings instead of adult trees. This is because the fire burned most of the vegetation acting as a “reset”, with the vegetation growing from scratch. Most species found were native, but besides silver wattle, we also found *Eucalyptus globulus* Labill., with more than 30% cover in areas with 10 follow-up controls of silver wattle. Although *E. globulus* isn’t legally considered invasive in Portugal (Decreto-Lei nº 92/2019), it clearly showed invasive behaviour in this study site (as is shows in several regions of the country, especially after the October 2017 fire, but not exclusively; Marchante *et al*., 2014) and as such it is also being controlled at PPSA (ICNF personal communication).Considering the persistent seed bank of silver wattle and that the seeds are stimulated by fire (Richardson and Kluge, 2008; Gibson *et al*., 2011; Passos *et al*., 2017), and knowing that in 2013 there was viable seeds accumulated in the soil seed bank despite most areas had no production of seeds since 2004/2005 (Rodrigues, 2014), it was expected that silver wattle presence increased, which happened (ICNF personal communication). However, this increase wasn’t visible in 2020, when this work was developed, because the ICNF technicians had already removed the numerous plants that had resulted from mass germination after the fire. In fact, field visits to the study site after the 2017 fire confirmed the presence of many silver wattle plants that had germinated after the fire (ICNF and E Marchante personal communication) (Figure 19). Furthermore, the number of work hours spent in each area per ha to make the follow-up control increased from 11 men/day (in 2017 before the fire) to 25 men/day (mostly in 2018, after the fire). In this context, the reduced number of silver wattle seeds found in the soil seed bank in this study was expected, both because production of seeds was prevented since 2004/2005 and because the fire of 2017 stimulated most of the remaining seeds to germinate. In fact, fire can be helpful in controlling silver wattle, as well as other fire adapted invasive plants, destroying adult plants able to produce seeds and stimulating seeds to germinate (Richardson and Kluge, 2008). However, this is particularly true when the fire is planned (prescribed burning) and proper follow-up measures anticipated and assured to quickly eliminate both the plants resulting from germination and resproutings of the adult plants; otherwise, fire can only worsen the invasion, since both the silver wattle that resprout and the seeds that germinate after fire can quickly reinvade the areas.

Figure 19. Silver wattle seedlings germinated after the fire in October of 2017 © Elizabete Marchante.

This study has some limitations, especially because some follow-up controls were made a few months before the field work, affecting the initial design and making the number of sampled areas uneven. Furthermore, one of the areas with 10 follow-up controls was dominated by tall eucalyptus, some seedlings and the rest was bare soil, which also biased the results a bit. Nevertheless, overall these limitations are not expected to have affected significantly the results, as the number of follow-up controls was always high and the results did not show significant differences between them.

Although these results are from only one study site, they have important implications for the management of invasive plants in general and they stress the crucial importance of assuring long-term follow-up control for a successful management of invasive species, especially the ones with long-lived seed banks and the ability to resprout by stumps and roots after fire. Additionally, more indirectly they also stress the relevance of acting after fire, preventing a fast reinvasion of the areas by invasive plants.

Conclusion

Results clearly showed that, by 2020, areas previously invaded by silver wattle at PPSA and subject to a continuous control program since 2004/2005 (with more than eight follow-up controls along time) show low presence of silver wattle and native vegetation is recovering to something close to the pre-invasion vegetation. However, these results are confounded by the effect of the 2017 fire, which burned all the vegetation, even if silver wattle is fire-adapted. In addition, aided by the fire in 2017 and the several follow-ups that prevented production of new seeds, the soil seed bank of silver wattle is now residual. The continuous reduction of silver wattle as the number of follow-up controls increases and the recovery of the native vegetation shows that controls have been effective and is a good result. This indicates that the effort and resources needed to assure further follow-up control is by now reduced. However, it needs to be kept in mind that only one silver wattle can start a new invasion, in four-five year it will produce seeds and these may either germinate or accumulate in the soil, starting a new cycle of invasion quite fast. Therefore, it is crucial to continue the follow-up controls and regular surveillance after no silver wattle is observed in the area in order to prevent new reinvasion, including after fire events. Only then it is possible to guarantee the successful control of silver wattle and the recovery of native vegetation.

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Annexes

Annex I. Hours of work spent controlling silver wattle (per hectare) in Paisagem Protegida da Serra do Açor by ICNF, from 2004/2005 to 2020, in each invaded area, considering the number of follow-up controls. Follow-up controls after 2018 were done after the October 2017 fire (in bold). All other follow-up controls, including in 2017, were made before the October fire. Source: ICNF

	Number of follow-up controls												
Area	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	200,0	55,0	18,8	25,0	20,0	21,3	55,0	7,5	15,0	4,4	3,8	**12,5**	**5,6**
2	96,4	73,6	29,6	15,7	30,4	16,8	3,8	3,4	**83,2**				
3	50,0	66,7	73,3	30,0	33,3	26,7	10,0	6,7	11,7	5,0	**13,3**		
4	110,0	35,0	70,0	20,0	5,0	25,0	3,8	3,8	2,5	3,8	2,5	**22,5**	
5	30,0	44,0	28,0	10,0	8,0	10,0	3,0	2,0	2,0	2,0	3,0	**12,0**	
6	55,6	34,4	33,3	15,6	4,4	13,9	10,0	7,8	2,8	2,8	1,7	**21,1**	
7	130,0	30,0	73,3	20,0	13,3	16,7	10,0	6,7	11,7	8,3	3,3	**8,3**	
8	18,0	14,0	25,0	8,0	6,0	5,0	3,0	5,0	3,5	4,0	6,5	**10,0**	
9	50,0	13,3	15,0	5,0	5,0	33,3	3,3	5,8	3,3	**15,8**			
10	153,3	86,7	90,0	113,3	80,0	43,3	33,3	15,0	**196,7**				
11	95,0	47,5	115,0	77,5	107,5	77,5	50,0	30,0	26,3	**42,5**			
12	80,0	100,0	126,7	130,0	80,0	101,7	63,3	36,7	23,3	**6,7**	**1,7**		
13	500,0	740,0	720,0	500,0	470,0	220,0	130,0	0,0	**20,0**	**5,0**			
14	42,5	52,5	77,5	62,5	85,0	33,8	42,5	17,5	**2,5**	**0,6**			
15	130,0	160,0	60,0	105,0	100,0	110,0	22,5	50,0	**5,0**	**1,3**			
16	28,9	62,2	42,2	5,6	27,8	22,2	15,6	0,0	**1,1**	**0,6**			
17	70,0	91,7	53,3	50,0	11,7	2,5	4,2	5,8	**1,7**				
18	140,0	110,0	32,5	25,0	30,0	12,5	8,8	3,8	**45,0**				

Annex II. List of plant species (54) identified in the sampling plots in Paisagem Protegida da Serra do Açor.

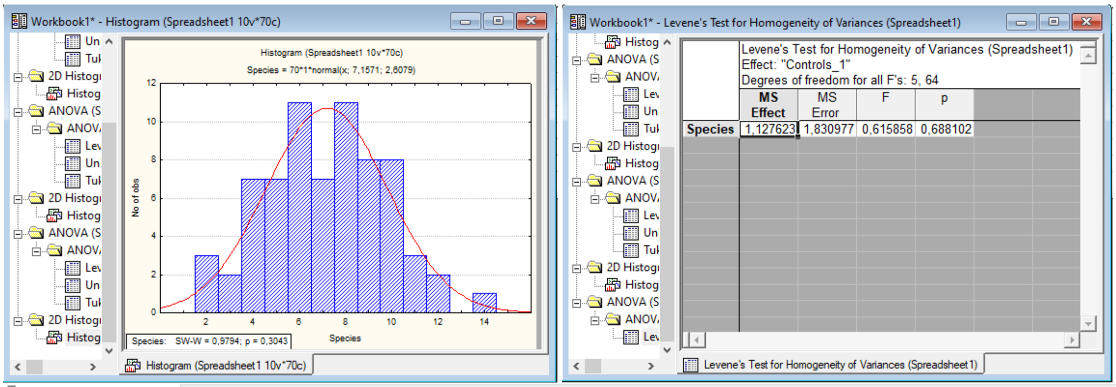
		Number of follow-up controls				
Family	**Plant species**	8	9	10	11	12
Asteraceae	*Andryala integrifolia*	x	x	x	x	
	Chamaemelum sp.		x	x		
	Filago pyramidata		x	x		
	Hispidella hispanica			x		
	Hypochaeris glabra	x			x	
	Lactuca sp.	x				
	Senecio sylvaticus	x	x		x	
	Taraxacum officinale	x	x			x
Boraginaceae	*Lithodora prostrata*	x	x			x
Brassicaceae	*Raphanus raphanistrum*	x	x		x	
Campanulaceae	*Campanula lusitanica*		x			
	Jasione montana		x			
Caryophyllaceae	*Silene muscipula*	x		x	x	
Cistaceae	*Tubelaria lignosa*				x	
Dennstaedtiaceae	*Pteridium aquilinum*	x	x	x	x	x
Ericaceae	*Arbutus unedo*		x		x	
	Erica andevalensis		x			
	Erica arborea	x	x		x	x
	Erica australis		x	x	x	
	Erica cinerea				x	x
Fabaceae	*Acacia dealbata*	x	x	x	x	x
	Cytisus striatus	x	x	x		
	Genista falcata				x	x
	Pterospartum tridentatum	x	x	x	x	x
	Trifolium pratense					x
	Ulex minor	x	x	x	x	
	Vicia angustifolia		x		x	
Fagaceae	*Quercus robur*	x	x			x
Hypericaceae	*Hypericum perforatum*	x	x		x	x
Lamiaceae	*Clinopodium vulgare*		x			x
	Lavandula stoechas		x	x		
	Rosmarinus officinalis		x			
Myrtaceae	*Eucalyptus globulus*	x		x		
Pinaceae	*Pinus pinaster*	x	x	x	x	x
Plantaginaceae	*Digitalis purpurea*	x	x	x	x	x
	Linaria triornithophora				x	
Poaceae	*Agrostis castellana*					x
	Anthoxanthum amarum	x	x	x	x	
	Avenula sulcata		x			
	Briza maxima	x	x	x	x	

	Briza minor		x			
	Dactylis glomerata	x			x	
	Festuca sp.	x				
	Holcus lanatus				x	
	Lolium temulentum		x			
	Micropyrum patens	x	x			
	Molinia caerulea				x	
Polygonaceae	*Rumex* sp.					x
Resedaceae	*Sesamoides suffruticosa*	x	x	x		x
Rosaceae	*Rubus ulmifolius*	x	x		x	x
	Sanguisorba verrucosa					x
Salicaceae	*Salix atrocinerea*	x				
Scrophulariaceae	*Scrophularia scorodonia*				x	
Valerianaceae	*Centranthus calcitrapae*	x	x	x	x	x
Xanthorrhoeaceae	*Simethis mattiazzi*				x	

Annex III. Statistical results

Vegetation

Species



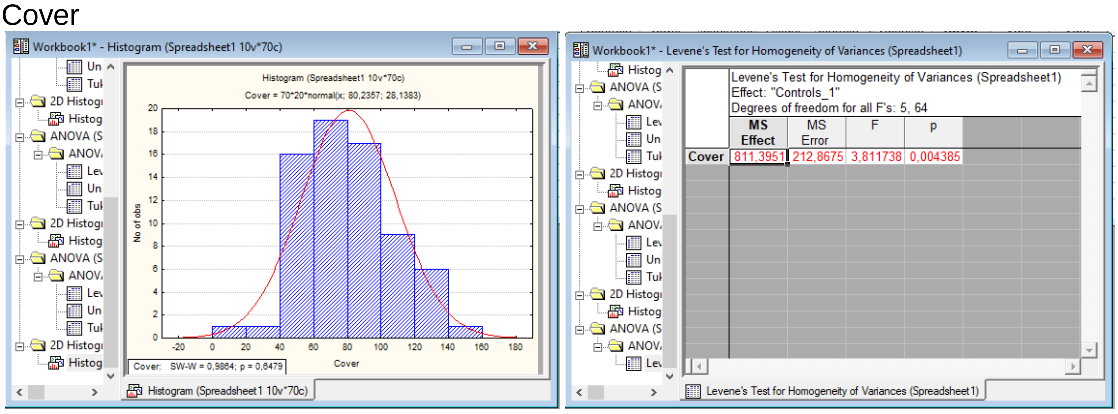
Median Test, Overall Median = 7,00000; Species (Spreadsheet1)
Independent (grouping) variable: Controls
Chi-Square = 10,47229 df = 5 p = ,0629

Dependent: Species	8	9	10	11	12	without *A. dealbata*	Total
<= Median: observed	4,00000	14,00000	8,00000	3,00000	1,00000	7,00000	37,00000
expected	5,28571	10,57143	5,28571	5,28571	2,64286	7,92857	
obs.-exp.	-1,28571	3,42857	2,71429	-2,28571	-1,64286	-0,92857	
> Median: observed	6,00000	6,00000	2,00000	7,00000	4,00000	8,00000	33,00000
expected	4,71429	9,42857	4,71429	4,71429	2,35714	7,07143	
obs.-exp.	1,28571	-3,42857	-2,71429	2,28571	1,64286	0,92857	
Total: observed	10,00000	20,00000	10,00000	10,00000	5,00000	15,00000	70,00000

Multiple Comparisons p values (2-tailed); Species (Spreadsheet1)
Independent (grouping) variable: Controls
Kruskal-Wallis test: H (5, N= 70) =14,49070 p =,0128

Depend.: Species	8 R:43,800	9 R:30,525	10 R:18,600	11 R:44,200	12 R:50,200	without *A. dealbata* R:37,167
8		1,000000	0,084384	1,000000	1,000000	1,000000
9	1,000000		1,000000	1,000000	0,797513	1,000000
10	0,084384	1,000000		0,073670	0,068758	0,381542
11	1,000000	1,000000	0,073670		1,000000	1,000000
12	1,000000	0,797513	0,068758	1,000000		1,000000
without *A. dealbata*	1,000000	1,000000	0,381542	1,000000	1,000000	

Cover



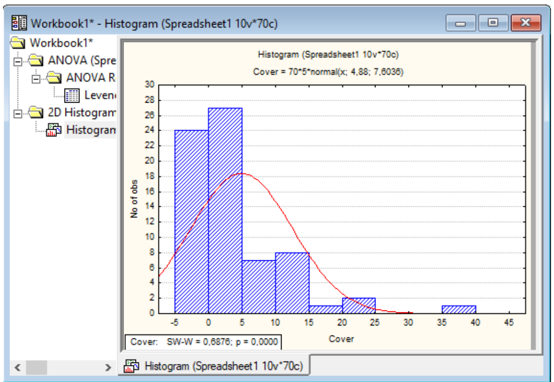
Median Test, Overall Median = 76,5000; Cover (Spreadsheet1)
Independent (grouping) variable: Controls
Chi-Square = 22,86667 df = 5 p = ,0004

Dependent: Cover	8	9	10	11	12	without *A. dealbata*	Total
<= Median: observed	1,00000	9,00000	9,00000	9,00000	3,000000	4,00000	35,00000
expected	5,00000	10,00000	5,00000	5,00000	2,500000	7,50000	
obs.-exp.	-4,00000	-1,00000	4,00000	4,00000	0,500000	-3,50000	
> Median: observed	9,00000	11,00000	1,00000	1,00000	2,000000	11,00000	35,00000
expected	5,00000	10,00000	5,00000	5,00000	2,500000	7,50000	
obs.-exp.	4,00000	1,00000	-4,00000	-4,00000	-0,500000	3,50000	
Total: observed	10,00000	20,00000	10,00000	10,00000	5,000000	15,00000	70,00000

Multiple Comparisons p values (2-tailed); Cover (Spreadsheet1)
Independent (grouping) variable: Controls
Kruskal-Wallis test: H (5, N= 70) =18,38971 p =,0025

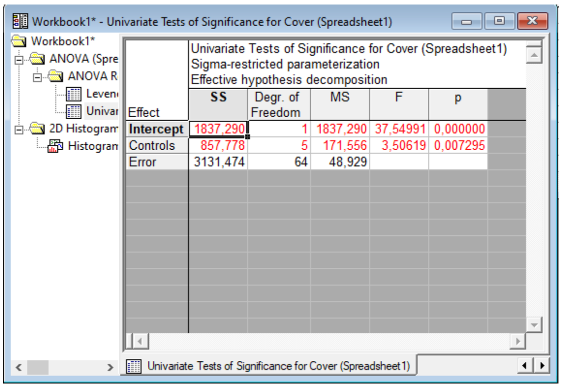
Depend.: Cover	8 R:52,350	9 R:37,725	10 R:19,700	11 R:23,200	12 R:30,800	without *A. dealbata* R:41,600
8		0,952857	0,005010	0,020412	0,797990	1,000000
9	0,952857		0,333048	0,980329	1,000000	1,000000
10	0,005010	0,333048		1,000000	1,000000	0,125864
11	0,020412	0,980329	1,000000		1,000000	0,401757
12	0,797990	1,000000	1,000000	1,000000		1,000000
without *A. dealbata*	1,000000	1,000000	0,125864	0,401757	1,000000	

Silver wattle
Cover

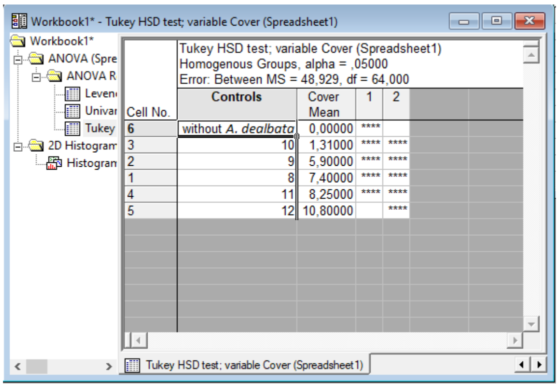


Levene's Test for Homogeneity of Variances (Spreadsheet1)
Effect: Controls
Degrees of freedom for all F's: 5, 64

	MS Effect	MS Error	F	p
Cover	124,8818	20,17463	6,190043	0,000097

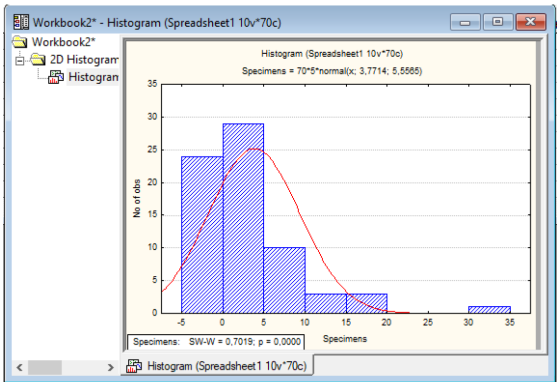

Univariate Tests of Significance for Cover (Spreadsheet1)
Sigma-restricted parameterization
Effective hypothesis decomposition

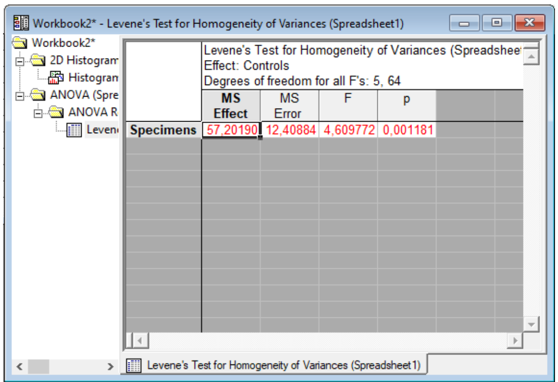
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1837,290	1	1837,290	37,54991	0,000000
Controls	857,778	5	171,556	3,50619	0,007295
Error	3131,474	64	48,929		


Tukey HSD test; variable Cover (Spreadsheet1)
Homogenous Groups, alpha = ,05000
Error: Between MS = 48,929, df = 64,000

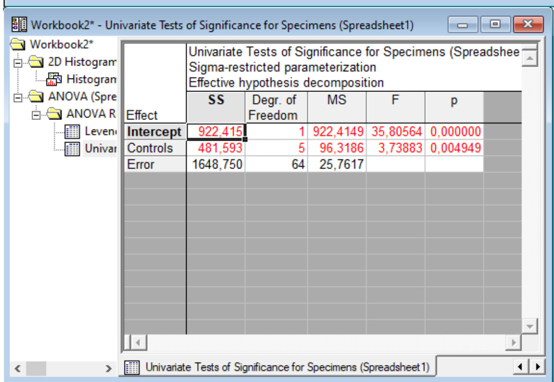
Cell No.	Controls	Cover Mean	1	2
6	without *A. dealbata*	0,00000	****	
3	10	1,31000	****	****
2	9	5,90000	****	****
1	8	7,40000	****	****
4	11	8,25000	****	****
5	12	10,80000		****

Specimens

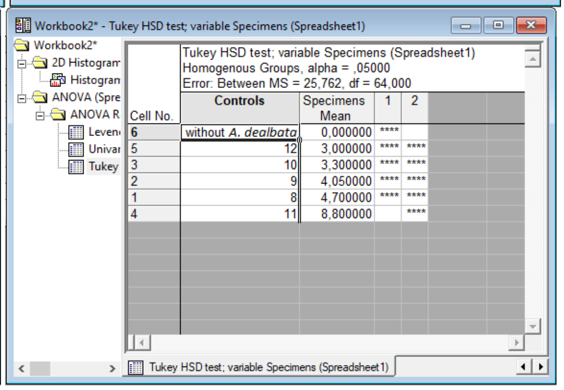



Levene's Test for Homogeneity of Variances (Spreadsheet1)
Effect: Controls
Degrees of freedom for all F's: 5, 64

	MS Effect	MS Error	F	p
Specimens	57,20190	12,40884	4,609772	0,001181

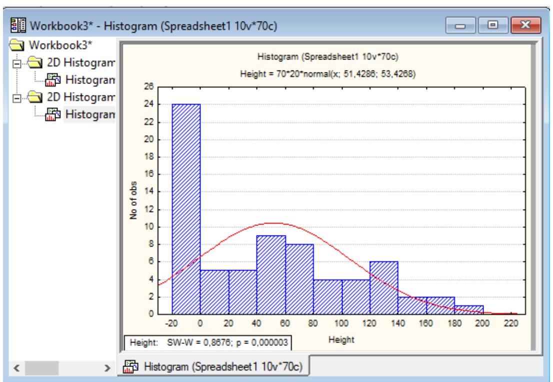

Univariate Tests of Significance for Specimens (Spreadsheet1)
Sigma-restricted parameterization
Effective hypothesis decomposition

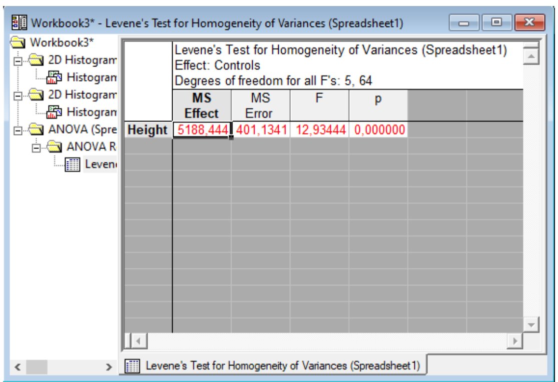
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	922,415	1	922,4149	35,80564	0,000000
Controls	481,593	5	96,3186	3,73883	0,004949
Error	1648,750	64	25,7617		


Tukey HSD test; variable Specimens (Spreadsheet1)
Homogenous Groups, alpha = ,05000
Error: Between MS = 25,762, df = 64,000

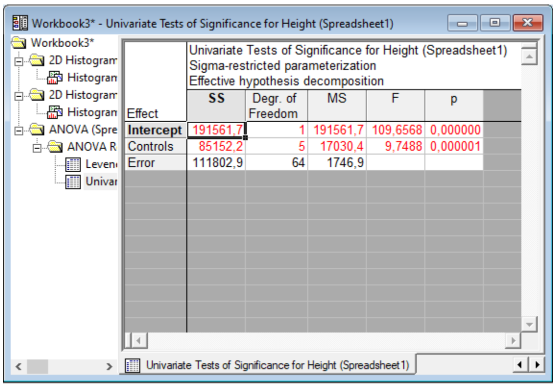
Cell No.	Controls	Specimens Mean	1	2
6	without *A. dealbata*	0,000000	****	
5	12	3,000000	****	****
3	10	3,300000	****	****
2	9	4,050000	****	****
1	8	4,700000	****	****
4	11	8,800000		****

Height



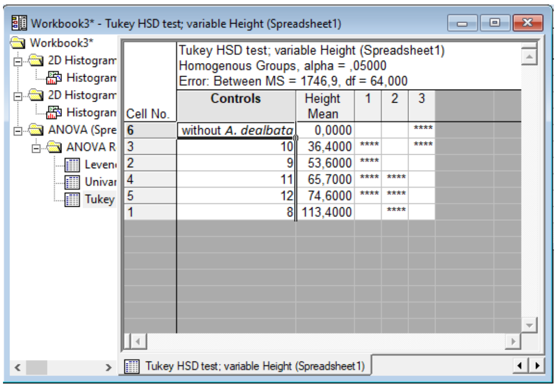

Levene's Test for Homogeneity of Variances (Spreadsheet1)
Effect: Controls
Degrees of freedom for all F's: 5, 64

	MS Effect	MS Error	F	p
Height	5188,444	401,1341	12,93444	0,000000



Univariate Tests of Significance for Height (Spreadsheet1)
Sigma-restricted parameterization
Effective hypothesis decomposition

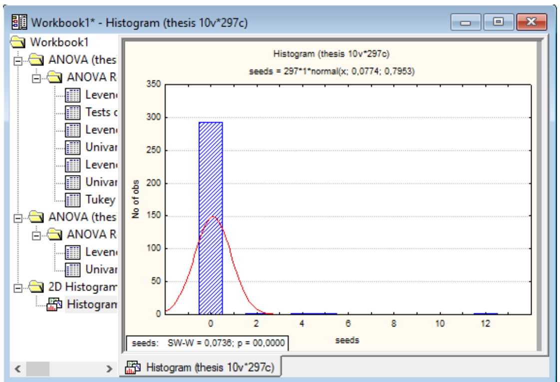
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	191561,7	1	191561,7	109,6568	0,000000
Controls	85152,2	5	17030,4	9,7488	0,000001
Error	111802,9	64	1746,9		

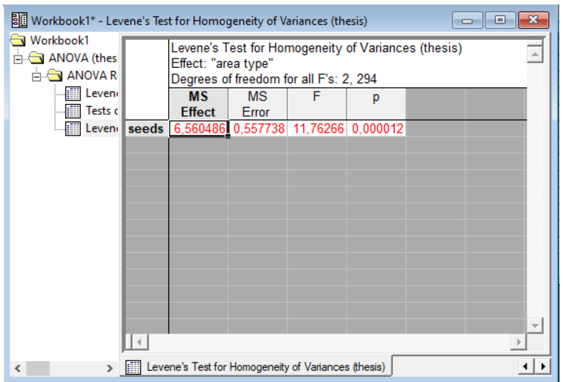


Tukey HSD test; variable Height (Spreadsheet1)
Homogenous Groups, alpha = ,05000
Error: Between MS = 1746,9, df = 64,000

Cell No.	Controls	Height Mean	1	2	3
6	without *A. dealbata*	0,0000			****
3	10	36,4000	****		****
2	9	53,6000	****		
4	11	65,7000	****	****	
5	12	74,6000	****	****	
1	8	113,4000		****	

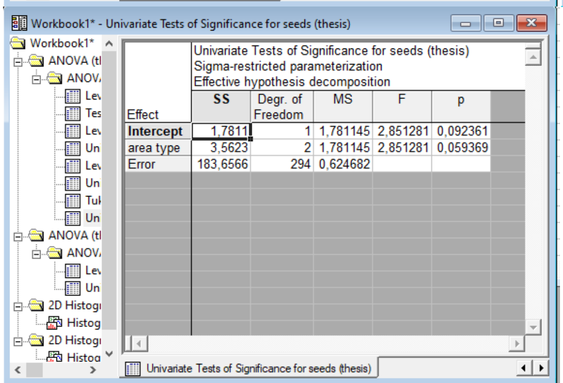
Seeds





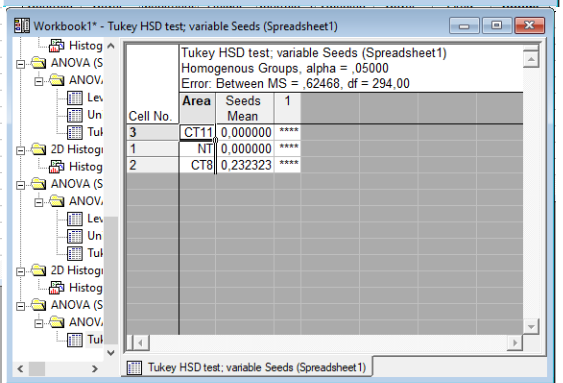
Levene's Test for Homogeneity of Variances (thesis)
Effect: "area type"
Degrees of freedom for all F's: 2, 294

	MS Effect	MS Error	F	p
seeds	6,560486	0,557738	11,76266	0,000012



Univariate Tests of Significance for seeds (thesis)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1,7811	1	1,781145	2,851281	0,092361
area type	3,5623	2	1,781145	2,851281	0,059369
Error	183,6566	294	0,624682		



Tukey HSD test; variable Seeds (Spreadsheet1)
Homogenous Groups, alpha = ,05000
Error: Between MS = ,62468, df = 294,00

Cell No.	Area	Seeds Mean	1
3	CT11	0,000000	****
1	NT	0,000000	****
2	CT8	0,232323	****