Invasion of Portuguese dunes by *Acacia longifolia*: present status and perspectives for the future

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ABSTRACT

Invasive species are one of the main threats to biodiversity worldwide, with serious impacts for the environment, economy and ecosystems services. Invasion ecology is a relatively recent field of increasing importance in environmental research and management, with a growing number of publications and new wide-reaching initiatives. Even so, many questions still need to be answered and more research is clearly needed. This work investigated several aspects of the invasion of a Mediterranean dune ecosystem by a *Leguminosae* tree, *Acacia longifolia* (Andrews) Willd.*,* aiming to contribute to filling some specific gaps in invasion *Ecology,* and providing practical and useful management options for managers.

The effects of *A. longifolia* at vegetation level, in areas with different invasion durations (stands invaded for more than 20 years, hereafter "long-invaded", compared to stands invaded after a summer fire in 1995 – hereafter "recently invaded") were compared, over a five-year period, with non-invaded situations, using multiple parameters. Results showed that the longer the invader is present in the system, the more pronounced are the impacts (*e.g.* decrease of plant cover, initial diversity and total species richness; fewer species shared with native areas; species traits altered) and the higher is the reinvasion potential due to germination of seeds of *A. longifolia*. Native dune ecosystems which were historically almost treeless were converted into highly modified systems, composed by almost mono-specific woodlands with a series of structural and functional changes which are dynamic and apparently tend to stabilize after several decades. The use of multiple parameters revealed that analyses including few, or only single figure parameters, concealed some of the changes taking place as invasions progress.

Seed ecology of *A. longifolia* was studied, including quantification of the seed rain (trials with seed traps), measurement of seed viability through time (burial experiments) and the extent of the soil seed bank (soil core extraction). Copious production of seeds was measured (ca. 12 000 seeds falling per m^{-2} annually), which concentrated mostly under the *A. longifolia* canopies. Although many seeds were lost both before and after entering the soil seed bank, seeds remaining in the soil were still numerous (ca. 1500 and 500 seeds m^{-2} in long- and recently invaded stands, respectively). Scarce seeds were found up to 7 m from the edge of stands, indicating that outside agents facilitated dispersal, and new invasion foci may arise if the right stimuli occur. This source of propagules enables *A. longifolia* to rapidly reinvade areas after disturbance (*e.g.* deliberate removal of plants, or fire). The number of seeds in the soil declined through time, with only 30% surviving after 6 years, with lower losses at greater depths. Germinability of buried seeds that survived was low (<12%) but viability was very high (>85%).

Recovery potential in long- and recently invaded stands was assessed using two distinct approaches: 1) soil seed banks were evaluated (seedling emergence method), comparing cleared plots (where both *A. longifolia* and its litter layer were removed) *vs.* uncleared plots; and 2) experimentally cleared plots were monitored, from 2002 to 2008 (a period that was longer than most removal studies), comparing *A. longifolia* removal with and without the underlying litter layer. Results of both experiments showed that the system is resilient, but less so in long-invaded areas, with many native plant species backing up autogenic recovery to a certain level. Nevertheless, many reappearing species were generalists and six years after clearing, several species and some traits typical from dunes were still missing (or scarce). Germination of *A. longifolia* seeds, as well as other exotics, impeded successful natural recovery, alerting that follow up interventions will be required. The seedling emergence method apparently gave an exaggerated impression of the extent of the degradation in actual field conditions, but was the only method that revealed *A. longifolia* seeds in non-invaded areas and allowed a rapid assessment of the ecosystem resilience without major clearing efforts. Long-term field monitoring of cleared sites provided a more detailed insight into the recovery potential of invaded areas, revealing more than twice the species found in the seedling emergence trials. However, the field trials were more expensive, time-consuming and took longer to furnish results. The removal of the thick litter layer (particularly in long-invaded areas) in field plots was cost-effective facilitating germination of more species and eliminating many *A. longifolia* seeds. Although distinct, both methods complemented each other.

The biological control agent *Trichilogaster acaciaelongifoliae* (Australian gall forming wasp), that targets seed reduction of *A. longifolia,* and additionally curtails its vegetative growth to some level, has been successfully used in South Africa for more than 20 years. It was therefore screened as a possible candidate for use in Portugal. Specificity tests, including 40 non-target plant species, gave promising results, indicating that *T. acaciaelongifoliae* can be a safe (and cost-effective) alternative to other control methods currently available in Portugal. Oviposition in non-choice tests was detected in three non-target species but subsequent trials in potted plants and surveys in the field (in Australia and South Africa) showed that galls only developed on *A. longifolia*. Although frequently considered as the most sustainable and environmentally friendly methodology around the world, biological control of invasive plants has only been used once in Europe, in early 2010. The implications of the results of the screening tests are discussed and an overview of the legal procedures needed to secure release of *T. acaciaelongifoliae* in Portugal is presented.

Synthesis: Despite the profound impacts of *A. longifolia* invasion on native vegetation and on seed banks, plant species recovery observed in the field and also soil seed bank studies showed that the

dune ecosystem is still resilient to some extent. Nevertheless, system degradation is striking and increases with time of invasion. The risk of reinvasion is high and long-lasting, due to the numerous long-lived seeds of *A. longifolia*. The results of this investigation emphasize the need for management actions that are sustainable in the long-term, and indicate that this can be achieved with biological control by an agent that primarily targets reduction of *A. longifolia* seed production.

RESUMO

A invasão por espécies exóticas é considerada uma das principais ameaças à biodiversidade à escala global, com graves impactes ambientais e económicos. O ramo da ecologia que se dedica ao estudo das invasões biológicas surgiu há pouco mais de meio século, mas tem adquirido uma notoriedade crescente, tanto a nível da investigação científica como a nível dos projectos de gestão dos problemas causados pelas espécies invasoras. O número de publicações especializadas, assim como os projectos de investigação e de gestão, tem aumentado de forma substancial e têm sido organizadas iniciativas diversas a todos os níveis de abrangência. A nível global, são exemplos o GISP (Global Invasive Species Programme), o GISIN (Global Invasive Species Information System) ou o ISSG (Invasive Species Specialist Group da IUCN, International Union for Conservation of Nature); a nível europeu podem referir-se a Estratégia Europeia para as Espécies Exóticas Invasoras, actualmente em discussão; várias comunicações oficiais da União Europeia sobre o problema e o projecto DAISIE (Delivering Alien Invasive Species Inventories for Europe, http://www.europe-aliens.org/); a nível nacional são exemplos a legislação sobre o tema – Decreto-Lei n.º 565/99, de 21 de Dezembro, e vários projectos de controlo de plantas invasoras em áreas protegidas, municipais e privadas.

Em Portugal, assim como em muitos outros locais do mundo (*e.g.* África do Sul, Chile, Nova Zelândia, Austrália, Espanha, Brasil, Israel), várias espécies do género *Acacia* são consideradas invasoras, sendo responsáveis por impactes negativos a nível da vegetação, disponibilidade de água, solo, ciclos biogeoquímicos, etc. *Acacia longifolia* (Andrews) Willd. (acácia-de-espigas), árvore de origem Australiana, é uma das espécies mais problemáticas em Portugal, sendo a sua expansão particularmente preocupante nos ecossistemas dunares. É neste contexto que se apresenta este estudo sobre a invasão da Reserva Natural das Dunas de S. Jacinto por *A. longifolia*, cujos objectivos gerais são: 1) avaliação dos impactes de *A. longifolia* a nível da vegetação, incluindo o banco de sementes, 2) estudo da ecologia das sementes de *A. longifolia*, 3) análise da capacidade de recuperação do ecossistema após remoção da espécie invasora, e 4) estudo do agente de controlo biológico *Trichilogaster acaciaelongifoliae* (vespa-australiana-formadora-de-galhas) como um método de controlo alternativo para usar em Portugal contra *A. longifolia*.

Para avaliar os impactes de *A. longifolia* a nível da vegetação (capítulo 2) monitorizaram-se, ao longo de cinco anos (2003-08), áreas invadidas há mais de 20 anos (acacial antigo), áreas invadidas após um incêndio no verão de 1995 (acacial recente) e áreas de vegetação nativa, não

invadidas. Analisaram-se comparativamente diversos parâmetros: riqueza específica, cobertura vegetal, índices de diversidade e de similaridade, curvas de abundância – dominância de diversos atributos funcionais e ecológicos e taxas de substituição de espécies. Os resultados mostraram que os impactes da espécie invasora são significativos ocorrendo, por exemplo, diminuição significativa da cobertura de plantas nativas, da diversidade, da equitabilidade e da riqueza específica nas áreas invadidas; adicionalmente, observou-se uma alteração profunda das espécies presentes implicando modificação dos atributos funcionais e ecológicos. Registou-se um agravamento dos impactes à medida que o tempo de invasão aumenta, *i.e.*, nas áreas de acacial antigo (*e.g.* diminuição mais acentuada da cobertura vegetal nativa nas áreas de acacial antigo e menor similaridade da composição de espécies entre áreas de acacial antigo e as áreas nativas). Por outro lado, verificou-se que o potencial de reinvasão, devido à germinação de sementes de *A. longifolia*, aumentou nas áreas invadidas há mais tempo. Os ecossistemas dunares em estudo, caracterizados no estado não invadido pela existência de diversas comunidades vegetais onde dominam espécies herbáceas, subarbustivas e arbustivas, foram substituídos por formações arbóreas, quase monoespecíficas, implicando uma série de transformações a nível da própria estrutura das comunidades e do seu funcionamento. Estas transformações são dinâmicas ao longo do tempo, conforme revelado pelas taxas de substituição de espécies, e tendem a estabilizar nas áreas invadidas há várias décadas. No conjunto, a análise dos vários parâmetros revelou também que o uso isolado de parâmetros expressos apenas por um valor (*e.g.* riqueza específica, cobertura vegetal) pode induzir em algumas interpretações menos correctas. A quantificação das alterações ocorridas devido à invasão é essencial, por exemplo, para complementar as análises de risco que podem fundamentar a classificação oficial de *A. longifolia* como "praga" no âmbito Europeu (Council Directive 2000/29/EC), ou para prioritizar áreas para gestão.

Com o objectivo de contribuir para o maior conhecimento da ecologia das sementes de *A. longifolia* (capítulo 3), procedeu-se à quantificação da queda de sementes (através da instalação de armadilhas de sementes), da viabilidade das sementes ao longo de um período de seis anos (através de experiências de enterramento de sementes a diferentes profundidades) e da extensão do banco de sementes acumulado no solo (através da recolha de amostras de solo). Os resultados obtidos mostraram que a produção anual de sementes de *A. longifolia* é elevada (12 000 sementes.m^{-2} em média); as sementes acumulam-se sobretudo debaixo das copas das árvores-mãe e apresentam, logo após a queda, germinabilidade (*i.e.* germinação sem receberem nenhum estímulo) da ordem dos 30-40% e viabilidade superior a 80%. Algumas sementes foram detectadas a 7 m da margem do acacial, sugerindo a intervenção de agentes de dispersão externos. Muitas sementes são, no entanto, perdidas, tanto antes como depois de entrarem no banco de sementes (*e.g.* devido a germinação, granivoria ou decomposição), resultando em valores médios de ca. 1500 e 500 sementes.m^{-2} acumuladas no solo de áreas de acacial antigo e de acacial recente, respectivamente. Apesar da perda significativa das sementes, as que são efectivamente acumuladas no banco de sementes são ainda numerosas sendo suficientes para reinvadir as áreas quando estas são sujeitas a alguma perturbação (*e.g.* acções de controlo, fogo). As sementes de *A. longifolia* acumuladas no banco de sementes das áreas de acacial antigo revelaram germinabilidades surpreendentemente baixas (< 5%) quando comparadas com as de bancos de sementes das áreas de acacial recente (ca. 65%). Um padrão semelhante, apesar da diferença ser menos acentuada, verificou-se para a viabilidade (ca. 70% *vs.* quase 100% em áreas de acacial antigo e de acacial recente, respectivamente). As sementes enterradas experimentalmente, foram desaparecendo ao longo do tempo e ao fim dos seis anos apenas ca. 30% das sementes foram recuperadas; as sementes enterradas mais profundamente (ca. 9 cm) foram recuperadas em maior quantidade. A germinabilidade destas sementes foi baixa (< 12%) mas a sua viabilidade foi muito elevada (> 85%). Estes resultados permitiram a caracterização do potencial de invasão de *A. longifolia* fundamental para o planeamento das acções de gestão da espécie invasora.

O potencial de recuperação de áreas de acacial antigo e de acacial recente foi avaliado recorrendo a duas abordagens distintas: 1. avaliação da constituição do banco de sementes (espécies nativas e exóticas) através do método de emergência de plântulas, em parcelas de onde *A. longifolia* foi removida juntamente com a camada de folhada e em parcelas não intervencionadas (capítulo 4)*;* 2. monitorização da recuperação florística desde 2002 a 2008 (constituindo um período mais longo do que maior parte dos estudos similares) em áreas de onde *A. longifolia* foi removida experimentalmente, com ou sem a camada de folhada acumulada à superfície do solo (capítulo 5). Os resultados das duas experiências revelaram que o sistema invadido ainda é resiliente, com muitas espécies de plantas nativas a suportar a recuperação autogénica parcial, apesar da resiliência ser menor em áreas de acacial antigo. No entanto, muitas das espécies nativas que colonizaram as áreas são generalistas (não exclusivas do sistema dunar e algumas até associadas a ambiente perturbados) e seis anos após a remoção da espécie invasora várias espécies típicas, e alguns atributos mais frequentes, das comunidades dunares eram muito escassos ou não (re) apareceram de todo. Por outro lado, a germinação de *A. longifolia*, assim como de outras espécies exóticas, ameaçaram o sucesso da recuperação alertando para a necessidade de continuidade das acções de gestão. Os resultados do método de emergência de plântulas, parecem indicar uma maior degradação do sistema comparativamente com a observada nas parcelas em campo;

contudo, este método revelou sementes de *A. longifolia* em áreas não-invadidas, o que não aconteceu nas parcelas em campo, e permitiu uma análise mais rápida da capacidade de recuperação do sistema sem a necessidade de um grande esforço/investimento em controlo. Por outro lado, a monitorização de longo - prazo em campo, permitiu informação mais detalhada do potencial de recuperação, revelando cerca do dobro das espécies detectadas no banco de sementes; no entanto, é uma metodologia mais dispendiosa, trabalhosa e que demora mais tempo até obter resultados. A remoção da camada de folhada (particularmente em áreas de acacial antigo) nas parcelas experimentais revelou-se vantajosa uma vez que, além de facilitar a germinação de mais espécies, contribuiu para eliminar muitas sementes de *A. longifolia*. Apesar de distintas, as duas abordagens complementaram-se permitindo uma análise mais completa da capacidade de recuperação do sistema. A análise do potencial de recuperação do sistema é fundamental para a definição das acções de gestão futuras, incluindo em relação à introdução de um agente de controlo biológico.

Os resultados dos capítulos anteriores revelaram que o sucesso das acções de controlo é rapidamente comprometido devido ao numeroso banco de sementes de *A. longifolia*, pelo que o controlo mecânico por si só não é uma alternativa eficaz. Uma opção a considerar para a redução da produção de sementes é a utilização de agentes de controlo biológico. Apesar do controlo biológico ser considerado frequentemente como uma das metodologias mais sustentável e "amiga-do-ambiente", a nível mundial, esta tecnologia aplicada a plantas invasoras foi usada apenas uma vez na Europa, com a introdução em 2010 do psilídeo *Aphalara itadori*, para controlo da sanguinária-do-Japão (*Fallopia japonica* (Houtt) R. Decr.) no Reino Unido. No caso da *A. longifolia*, o agente de controlo biológico *Trichilogaster acaciaelongifoliae* é reconhecido como sendo mono-específico (*i.e.* capaz de completar o seu ciclo de vida apenas em *A. longifolia*) e reduz significativamente a produção de sementes de *A. longifolia*, além de diminuir, até certo nível, o seu crescimento vegetativo. *Trichilogaster acaciaelongifoliae* é usado com sucesso na África do Sul, onde foi introduzido há mais de 20 anos, e foi testado como uma alternativa para usar em Portugal (capítulo 6). Os resultados dos testes de especificidade realizados, incluindo 40 espécies de plantas não-alvo, indicaram que *T. acaciaelongifoliae* pode ser uma alternativa segura (e mais económica) aos métodos de controlo de *A. longifolia* actualmente disponíveis em Portugal. Testes de oviposição sem-escolha revelaram deposição de ovos em três espécies não-alvo. Fases subsequentes das experiências, em plantas envasadas (em laboratório) e observações em campo (na Austrália e na África do Sul), mostraram que apenas se desenvolveram galhas, *i.e.,* o agente conseguiu concluir o seu ciclo de vida, em *A. longifolia*. Os dados dos estudos atrás referidos (nomeadamente a

quantificação dos impactes e a caracterização da produção, viabilidade e banco de sementes de *A. longifolia*) são indispensáveis para avaliar futuramente o grau de sucesso do agente de controlo biológico, caso a sua libertação venha a ser autorizada. Discutem-se as implicações da utilização desta metodologia e apresenta-se um resumo dos procedimentos legais necessários antes da sua libertação.

Síntese: apesar da invasão por *A. longifolia* ter impactes profundos na vegetação nativa e no próprio banco de sementes, a recuperação das espécies vegetais observada em campo e prevista através da análise do banco de sementes, revelaram que o sistema dunar em estudo ainda é resiliente. No entanto, a degradação do sistema dunar é significativa e agrava-se com o aumento do tempo de invasão. Acresce que esta degradação se repercute também num risco de reinvasão que é elevado devido ao banco de sementes da espécie invasora que, além de numeroso, tem viabilidade longa. Os resultados deste trabalho reforçam a necessidade de combater o problema de invasão por *A. longifolia* com acções de gestão de longo-prazo. Adicionalmente, indicam que uma das metodologias de controlo possíveis para *A. longifolia* é a utilização do agente de controlo biológico *T. acaciaelongifoliae*, o qual se prevê que diminua a produção de sementes de forma significativa e contribua para reduzir o seu crescimento vegetativo, de forma ambientalmente segura e economicamente sustentável.

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LIST OF PUBLICATIONS AND MANUSCRIPTS:

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Chapter 2, submitted to *Conservation Biology*: (p. 43)

Marchante, H.; Freitas, H. & Hoffmann, J. H. Long term changes in vegetation structure of a Mediterranean coastal dune invaded for different durations by *Acacia longifolia*

Chapter 3, published as: (p. 67)

Marchante, H., Freitas, H. & Hoffmann, J.H. 2010. Seed ecology of an invasive alien species, *Acacia longifolia* (Fabaceae), in Portuguese dune ecosystems. *American Journal of Botany* **97(11)**: 1–11.

Chapter 4, published as: (p. 91)

Marchante, H., Freitas, H. & Hoffmann, J.H. 2010. The potential role of seed banks in the recovery of dune ecosystems after removal of invasive plant species. *Applied Vegetation Science*, DOI: 10.1111/j.1654-109X.2010.01099.x, 1–14.

Chapter 5, submitted to *Journal of Applied Ecology*: (p. 117)

Marchante, H.; Freitas, H. & Hoffmann, J. H. Post-clearing recovery of coastal dunes invaded by *Acacia longifolia*: is duration of invasion relevant for management success?

Chapter 6, partially published as: (p. 139)

Marchante, H., Freitas, H. & Hoffmann, J.H. 2010 Assessing the suitability and safety of a well-known bud-galling wasp, *Trichilogaster acaciaelongifoliae*, for biological control of *Acacia longifolia* in Portugal. *Biological Control,* doi: 10.1016/j.biocontrol.2010.11.001.

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[1] References and figures formatting in each chapter were kept according to the rules of journals where they were published/ submitted.

Part I

Chapter 1

General Introduction

This introduction is a brief state of the art of key issues on biological invasions (*e.g.* terminology issues, species characteristics, invasive alien species impacts), also focusing in some specific topics related to the five main chapters of this thesis (chapters 2 to 6). In order to make the geographic context of the problem, the situation is characterized in Europe and then in Portugal. A particular importance is then given to IAS management as one of the underlying goals of this work is to provide useful information for managers. Finally, the species and the ecosystem in study are characterized. Subjects that are depicted in detail in each of the five main chapters were in most cases excluded in order to avoid much repetition.

INVASIVE SPECIES: AN OVERVIEW

State of the art

Biological invasions mediated by human activities are a huge source of change in ecosystems, with escalating impacts, both ecological and economic, worldwide (Brooks *et al.*, 2004; Rejmánek *et al.*, 2005; Mason, French & Russell, 2007; Yelenik, Stock & Richardson, 2007; Batten, Scow & Espeland, 2008; Marchante *et al.*, 2008b; Gaertner *et al.*, 2009; Jäger, Kowarik & Tye, 2009; Pejchar & Mooney, 2010; Pyšek & Richardson, 2010; Vilá *et al.*, 2010), often resulting in an “invasional meltdown” [1] (sensu Simberloff & Von Holle, 1999). The origin of biological invasions is lost in time with some authors pointing to Late Devonian (Stigall, 2010). Nevertheless, it has increased consistently under human-mediation, reaching a peak in the second half of the 20th century (Pyšek & Richardson, 2010) when it gained “formal” recognition in the late 1950s with the pioneering work of Charles Elton (Elton, 1958), often considered as the “father of Invasion Biology” (Richardson & Pyšek, 2008). Scientists, politicians and Global Organizations (Ministério do Ambiente, 1999; Millennium Ecosystem Assessment, 2005; Commission of the European Communities, 2008; ISSG/IUCN, 2008; Pyšek & Richardson, 2010; TEEB, 2010) all recognize that invasive species damage ecosystem services, disrupt human well-being and threaten biodiversity. Ecosystem services affected by invasive species include supporting (*e.g.* alteration of succession patterns and soil and nutrient cycling), provisioning (*e.g.* threats to native species, alteration of genetic resources), regulating (*e.g.* changing pollination services and fire regimes) and cultural services (*e.g.* effects on ecotourism, changes in perception of landscape) (Millennium Ecosystem Assessment, 2005; Vilá *et al.*, 2010). The alterations that occur at one trophic level may have repercussions into several other trophic levels (*e.g.* alterations in plant communities affect herbivores and parasitoids which are interlinked in food webs) amplifying the more frequently measured impacts of invasive alien species (IAS) (Carvalheiro, Buckley & Memmott, 2010).

Invasive species are reported to rank as the second agent of species endangerment and extinction (Pejchar & Mooney, 2010). Nevertheless, and despite the unquestionably extensive and profound impacts of IAS, there is no quantitative data that supports this universal ranking. Such level of threat has been justified for particular systems and taxonomic groups, where invasive species may even be

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Invasional meltdown refers to the interactions among invaders that accelerate invasions and amplify their effects on native communities the predominant threat (Miller, 1989; Wilcove *et al.*, 1998). On a global scale, it is more correct to include biological invasions amongst the five mains causes of biodiversity decline (Millennium Ecosystem Assessment, 2005).

Invasive alien species (see Table 1.1 for definitions) are found amongst all living organisms, including many plants, animals and microrganisms (Elton, 1958; Vitousek, 2001; Perrings, Mooney & Williamson, 2010). Some of those species are "born" invaders (*i.e.* without major limitations on their adaptation to new environments) others are "made" invaders (*i.e.* evolve after being introduced in the new environment in order to adapt and become more fit) (Pyšek & Richardson, 2010). This work focuses on invasive plants, in particular the *Leguminosae* tree *Acacia longifolia*.

Table 1.1 Main concepts and definitions used in plant invasion biology (source Richardson *et al.*, 2000).

Concept	Definition
Exotic, alien, aloctonous, non-native, non-indigenous	Plant taxa whose presence in a given area is due to introduction, intentional or accidental, as a result of human activity.
Casual, occasional escape, transient	Subset of alien plants that may flourish, and even reproduce occasionally in an area, but which do not form self-replacing populations, and which rely on repeated introductions to persist.
Naturalized, sub-spontaneous	Subset of alien plant that reproduce consistently and sustain populations over many life cycles without direct intervention by humans (or in spite of human intervention); often recruit offspring freely, usually close to adult plants, and do not necessarily spread into natural, semi-natural or human-made ecosystems.
Invasive, environmental weed	Subset of naturalized plants that produce reproductive offspring, often in very large numbers, at considerable distances from parent plants (approximate scales: > 100 m; < 50 years for taxa spreading by seeds and other propagules; > 6 m/3 years for taxa spreading by roots, rhizomes, stolons, or creeping stems), and thus have the potential to spread over a considerable area; frequently adversely affecting native biodiversity and/or ecosystem functioning.
Transformer	Subset of invasive plants which change the character, condition, form or nature of ecosystems over a substantial area relative to the extent of that ecosystem.
Weeds, plant pests, harmful species	Plants (not necessarily exotic) that grow in sites where they are not wanted by humans and which usually have detectable economic or environmental effects.

Invasive alien species are a small fraction of exotic species that are increasingly moved around the world, intentionally or accidentally, crossing geographic barriers (and then a series of environmental and other barriers, see Figure 1.5 and Richardson *et al.* (2000) for more details) that had once limited their distribution to their natural ranges. Often, such species are moved without carrying the natural enemies that in their native environment are one of the factors contributing to keeping them in check - enemy release hypothesis ERH (Keane & Crawley, 2002). ERH when associated with some species traits, *e.g.* prolific seed production, efficient mechanisms of dispersal, high growth rates, plasticity, altogether facilitate a plant species becoming invasive. Some invasive species are able to significantly modify the ecosystems over a substantial area; these are named transformers (Richardson *et al.*, 2000).

Some of the characteristics of the species, *e.g.* prolific production of seeds which accumulate in long-lived, viable seed banks, are particularly important, because besides being responsible for initial invasion they ensure persistence into the future by allowing resurgence after control efforts or other forms of disturbance. Whether a species is invasive or not depends not only on the characteristics of the species but also on the characteristics of the new environment itself including factors such as resources availability, levels of antagonist species (*e.g.* predators, herbivores, pathogens) and intactness of the system. Propagule pressure (*i.e.* introduction effort), other human activities (reflected in variables such as Human wealth and demography), and intensity of disturbance (Essl *et al.*, 2010; Perrings, Mooney & Williamson, 2010; Pyšek *et al.*, 2010) can also play an important role in the process of whether or not a species will become invasive.

Although some ecosystems are intrinsically more susceptible to invasion than others, disturbance, fragmentation and changes in land-use are known to greatly increase ecosystems vulnerability to invasion, even with the same activity affecting different ecosystems types in distinct ways (Pyšek, Chytrý & Vojtech, 2010). Other authors (Blossey & Notzold, 1995) noted another factor, expressed as the EICA (Evolution of Increased Competitive Ability) hypothesis, according to which, species, after introduced into a new location, evolve and adapt to the new environment being able to reallocate resources in order to gain competitive advantage. This hypothesis makes it more difficult to predict which species will became invasive because unpredicted evolution, following introduction, can make a difference.

The time lag between the introduction of a species and the revelation of its invasive behaviour can be extremely variable. Even so, a recent study highlights that many of the most problematic invasive species (for all major taxonomic groups) are species that were introduced several decades ago, calling it an “invasion debt” and alerting to the fact that current human activities (including globalization and economic growth) will have their consequences on the extent of biological invasions several decades into the future (Essl *et al.*, 2010).

The economic and ecological dimensions of biological invasions are interconnected and are far reaching. The changes in ecosystems that frequently increase their susceptibility to invasion impacts (*e.g.* disturbance, habitat fragmentation) are frequently economically mediated; and the resulting ecological impacts (normally considered as externalities to the economic activities and as such not taken into account by its promoters) have direct implications on the "natural capital" with repercussions which are manifest as economical problems. An increasing number of regional trade agreements (more than 420 reported to the World Trade Organization) aim to reduce barriers to movement of commodities and people, associated with increased trade, transport and travel all over the world (as a result of globalization). Although these agreements have many benefits for human well-being, they are increasingly facilitating the introduction of more species (some new, others already problematic), and consequently increasing probabilities of establishment and dispersal of such species (Perrings, Mooney & Williamson, 2010). Additional problems arise from the ever-increasing online trade, which facilitates unregulated movement of species, including invasives, to virtually every part of the globe (Derraik & Phillips, 2010).

Economic impacts of IAS, measured so far, include mainly management costs and also losses to provisioning ecosystem services (Pimentel, Zuniga & Morrison, 2005; Pejchar & Mooney, 2010; Vilá *et al.*, 2010). Some of these costs could be partially balanced /attenuated if probability of success of costly management options was first evaluated, in a systematic manner, in order to weigh the possibility of "doing nothing" against the management actions and thus avoiding some of the huge funding efforts in systems without clear chances of success.

Besides environmental and economic effects of IAS, cultural impacts, centred in human uses and traditions, are quite diverse being possible to find invasive species culturally impoverishing (*i.e.* imply loss or replacement of culturally important native species), culturally enriching (*i.e.* augment cultural traditions), or culturally facilitating (*i.e.* continuity and reformulation of traditional ethnobiological practices) (Pfeiffer & Voeks, 2008). When considering management of invasive species, all these dimensions of impacts have to be balanced in an integrative way.

Invasive alien species in Europe - where do we stand?

"*Invasive alien species are currently among the most urgent nature conservation issues to be faced in the European Union and many important steps are being undertaken to develop an adequate strategy to deal with this problem*" in Scalera (2010)

Europe in general was perceived until recently as being a major source of invasive species for other regions of the world but not receiving many species nor having major problems with invasive species. This concept has changed greatly after a continent wide assessment made by the DAISIE project (http://www.europe-aliens.org/). DAISIE revealed over 11 000 alien species reported including terrestrial plants, invertebrates and vertebrates, freshwater and marine flora and fauna. Of these, over half of the species are terrestrial plants. A recent assessment aiming to foresee the distributions of invasive species across habitats in Europe, predicted that the highest levels of invasion occur in disturbed habitats (agricultural, urban and industrial land-cover classes), while low levels occur in natural and semi-natural grasslands and woodlands, and the lowest levels in sclerophyllous vegetation, heathlands and peatlands (Chytrý *et al.*, 2009). The same study predicted that lowland areas of the temperate zone of western and central Europe have high levels of invasion while boreal zone, mountain regions across the Europe and Mediterranean region (except its coastline, river corridors and areas with irrigated agricultural land) have low levels of invasion.

A rough assessment of impacts, continental wide, estimated that financial costs are over €10 billion annually (Hulme *et al.*, 2009), including mainly management costs, such as eradication, control, monitoring and environmental education programs, and also losses to provisioning ecosystem services (Vilá *et al.*, 2010). Approximately 300 projects addressing IAS have been funded by the European Commission (EC), over the last 15 years, with a total budget exceeding €132 million (Scalera, 2010). These included projects financed by LIFE Programme and Framework Programmes for Research and Technological Development (FPs), with a higher investment on research than on management actions. Terrestrial plants and invertebrates include the majority of species with impacts although economic and environmental impacts are known for only 11-13% of the species (documented ecological impacts for 1094 species and economic impacts for 1347 species, including all taxonomic groups (Vilá *et al.*, 2010).

Invasive plants, in particular, have more ecological impacts reported in Europe than economic impacts, because of difficulty in to attributing market-based costs to ecological impacts measured.

Although there are several commitments and agreements underpinned by international European organizations (*e.g.* EPPO and the EU itself), a specific European strategy and dedicated financial instrument targeting IAS is still missing (Scalera, 2010). Little has translated into action (*e.g.* prevention of new introductions, successful management actions) and IAS problems keep increasing (Hulme *et al.*, 2009). Several issues make it difficult for Europe to achieve such action at a continental scale; e.g., the single market policy that removed the barriers to movement of commodities and people, the lack of a “European species blacklist” that would have to consider regional lists (as many species are native in some European countries while exotic in others *e.g. Rhododendron ponticum* L. which is native in Portugal and invasive in the UK) and climatic zones (as some species are invasive in some areas but are climatically constrained in others). Lack of information for the majority of the species also complicate efforts because would be needed for complete risk assessments that would allow listing some of the worst species as “official pests” (Council Directive 2000/29/EC).

Portugal in more detail

There are over 820 exotic species in Portugal, including terrestrial and aquatic animals and plants and fungi; exotic terrestrial plants and insects are the most numerous (Table 1.2) (DAISIE, 2008). Amongst these, many are invasive and perceived as widespread and causing major ecological (Almeida & Freitas, 2001; Marchante, 2001; Campos, Rocha & Tavares, 2002; Aguiar *et al.*, 2005; Anastácio, Parente & Correia, 2005; Fernandes, 2008; Marchante *et al.*, 2008b, a; Sousa *et al.*, 2008; Hellmann *et al.*, 2010) and economic impacts (Scalera & Zaghi, 2004). Invasive species are legally recognized since 1999 by Decreto-Lei n.º 565/99 de 21 de Dezembro, which forbids the use of species that are listed as invasive. The list of species needs to be updated as several problematic invasive species are not yet included. Controversially, other legal instruments seem to neglect the problem, *e.g.* some exotic invasive species such as *Paspalum* spp. have a conservation status due to be considered indicators of habitats protected by Natura 2000 Network (Decreto-Lei nº 140/99 de 24 de Abril – Anexo B-1; Directiva 92/43/CEE – Anexo I).

Table 1.2 Exotic species registered in Portugal, distributed by major taxonomical categories (based on information from DAISIE (2008); Almeida and Freitas (2006) and our own records on plant species).

Terrestrial fungi		**Aquatic inland**		**Terrestrial plants**		**Terrestrial invertebrates**				**Terrestrial vertebrates**			**Total of exotic species**
Fungi	*Chromista*	*Crustacea*	*Magnoliophyta*	*Magnoliophyta*	*Bryophyta*	*Other arthropods*	*Araneae*	*Nematoda*	*Insecta*	*Aves*	*Reptilia*	*Mammalia*	
13	3	2	2	557	2	1	7	3	208	24	3	2	**827**

Some of the more widespread and injurious invasive animals in Portugal are the Louisiana crayfish (*Procambarus clarkii*), pinewood nematode (*Bursaphelenchus xylophilus*) and the Asian clam

(*Corbicula fluminea*) (Anastácio, Parente & Correia, 2005; Naves, Camacho & de Sousa, 2006; Sousa *et al.*, 2008). Invasive plants are more numerous than animals, and probably better studied, with invasive plant species publications becoming increasingly more frequent (Marchante, Marchante & Freitas, 2003; Peperkorn, Werner & Beyschlag, 2005; Aguiar, Ferreira & Albuquerque, 2006; Almeida & Freitas, 2006; Aguiar *et al.*, 2007; Ferreira, Máguas & Martins-Loução, 2007; Rodríguez-Echeverría, Crisóstomo & Freitas, 2007; Fernandes, 2008; Marchante *et al.*, 2008b, 2009), including several studies quantifying impacts at different levels. Compared with other European countries, Portugal has more invasive tree species than most of the northern countries (DAISIE, 2008), which sometimes results in more extreme changes at the landscapes.

Over 550 exotic plant species (including only casuals, naturalized and invasive) are considered to be introduced in Portugal (Almeida & Freitas, 2006), being rather well characterized regarding introduction time and pathways, native range and taxonomy (Almeida, 1999; Marchante, Freitas & Marchante, 2008). Their native ranges include regions all around the world, with over 70 species originating from each of the Americas', Eurasia; other regions of Europe and Africa (Almeida, 1999). Nevertheless, Australasia despite being the native range of fewer species, is home to a substantial proportion of the most problematic species in Portugal, such as *Acacia* spp., *Hakea* spp. and *Pittosporum undulatum* Vent. (Santo & Arsénio, 1999; Campos, Rocha & Tavares, 2002; Fernandes, 2008; Marchante, Freitas & Marchante, 2008).

Most of the exotic plant species (ca. 50%) were introduced as ornamentals (Figure 1.1), with agri/horticulture also accounting for a substantial number of introductions. This pathway is still "active" with new species being introduced every year. About 1/6 of the species were accidentally introduced, which corresponds to numerous cases of agricultural weeds whose seeds were acquired unintentionally with crop seeds (Almeida & Freitas, 2000).

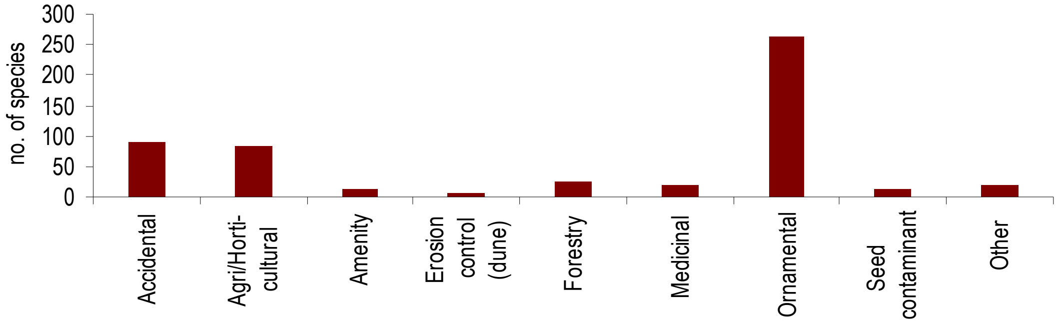


Figure 1.1 Introduction pathways of the exotic plant species recorded in Portugal. Some of the species (ca. 50) were introduced by more than one pathway and are included more than once in the graphic. Species strictly limited to cultivation are nit included.

Amongst the vast taxonomic diversity (110 families) of the exotic plant species present in the country, several families are absent from the Portuguese native flora (*e.g. Oxalidaceae, Proteaceae, Pittosporaceae*) and many species are grouped in just a few families - *Asteraceae, Fabaceae* and *Poaceae*, each contributing with over (or close to) 10% of the species (Almeida & Freitas, 2006).

Exotic plant species occur in a wide range of Portuguese habitats with some of the more humanized and disturbed habitats (I1 and J4 – Table 1.3) (Almeida, 1999; H. Marchante *et al., unpublished data*) having more species, reflecting the pattern of plant invasions distribution predicted for Europe (Chytrý *et al.*, 2009). Many species are also present in other habitats, including the coastal sand dunes (48 species) and littoral zone of inland water bodies (70 species) (Moreira *et al.*, 2002; Aguiar, Ferreira & Albuquerque, 2006; Aguiar *et al.*, 2007; Marchante, Freitas & Marchante, 2008).

Table 1.3 Habitats where exotic plant species are present in Portugal, according to the EUNIS classification (Almeida, 1999; H. Marchante *et al, unpublished data*). Ca. 200 species occur in more than one habitat.

EUNIS code	Habitat description	No. of species
I1	Arable land and market gardens	164
J4	Transport networks and other constructed hard-surfaced areas	164
C3	Littoral zone of inland surface waterbodies	70
G	Woodland and forest habitats and other wooded land	57
B1	Coastal dune and sand habitats	48
J2	Low density buildings	43
FA	Hedgerows	36
G4	Mixed deciduous and coniferous woodland	27
J6	Waste deposits	20
F9	Riverine and fen scrubs	19
H3	Inland cliffs, rock pavements and outcrops	19
H5	Miscellaneous inland habitats with very sparse or no vegetation	18
B3	Rock cliffs, ledges and shores, including the supralittoral	18
D1	Raised and blanket bogs	15
I2	Cultivated areas of gardens and parks	12
J1	Buildings of cities, towns and villages	12
H2	Screes	10
G3	Coniferous woodland	10

The areas that have registered the introduction of most exotic plant species are the heavily populated coastal areas, namely Estremadura (335 species), Beira Litoral (255 species) and Douro Litoral (208 species) (Figure 1.2), which confirms the strong human factor associated with IAS problem.

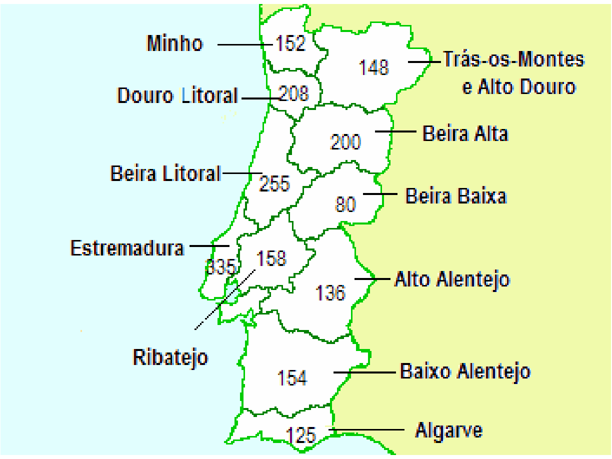


Figure 1.2 Number of exotic plant species recorded in each province of Portugal (based on Almeida, 1999). Despite provinces are no longer recognised as administrative regions in Portugal they were used because data from several Flora refer to these regions.

The attribution of a status to the exotic species is neither consensual, nor completely objective, nor static in time. Even so, an exercise (H. Marchante *et al.*, *unpublished data*) aiming to classify the exotic plant species occurring in Portugal reveals that most of the species appear as naturalized or casuals and ca. 10% reveal invasive behaviour (Figure 1.3).

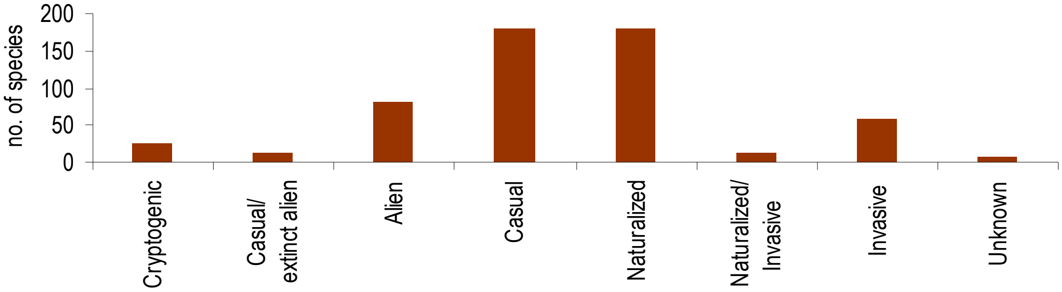


Figure 1.3 Invasive status of the exotic plant species in Portugal. Species strictly limited to cultivation are not included.

Of these, 29 are considered invasive by law, including most of the more problematic species, *e.g. Acacia dealbata, Ailanthus altissima*, *Carpobrotus edulis*, *Eichhornia crassipes* or *Hakea sericea* (Figure 1.4); other species not yet listed as invasive in the legislation (*e.g. Cortaderia selloana*, *Opuntia maxima*), already reveal vast distributions along the country (Marchante, Freitas & Marchante, 2008).

Acacia dealbata Link

Acacia melanoxylon R.Br.

Acacia retinodes Schlecht

Acacia saligna (Labill.) H. L. Wendl.

Acacia longifolia (Andrews) Willd.

Acacia mearnsii De Wild.

Carpobrotus edulis (L.) N. E. Br.

Cortaderia selloana (Sch & Sch) Asch. & Graebn

Robinia pseudoacacia L.

Conyza spp.

Azolla filiculoides Lam.

Opuntia maxima Miller

Tradescantia fluminensis Velloso

Ipomoea acuminata (Vahl) Roemer & Schult

Myriophyllum aquaticum (Velloso) Verdc

Pittosporum undulatum Vent.

Hakea salicifolia (Vent.) B.L. Burtt

Hakea sericea Schrader

Ailanthus altissima (Mill.) Swingle

Eichhornia crassipes (Mart.) Solms

Figure 1.4 Some of the more problematic invasive plant species in Portugal.

Under a panorama of so many exotic and/or invasive species, it is particularly important to be aware of what are the management options.

How to manage biological invasions?

The problem of biological invasions is complex. It integrates many factors (*e.g.* climate change, globalization, species and habitats characteristics, human actions), creates conflict of interests (*e.g.* World Trade Organization, ornamental commerce, wood industries *vs.* restrictions to species utilization due to their invasiveness), involves technical challenges (*e.g.* control of online trade, finding effective control strategies, accurate taxonomic identification) and is aggravated by a generalized lack of public awareness (*e.g.* who can have active roles, either promoting new introductions or controlling existing invasive species), often amongst stakeholders and managers (Andreu, Vilá & Hulme, 2009; Pyšek & Richardson, 2010). As such, management of biological invasions demands well planed, integrative actions, designed for several years, frequently even decades, with the underlying need of continuous funding (Wittenberg & Cock, 2005). A fundamental factor that has to be considered as a baseline on IAS management is habitat disturbance which frequently works as a facilitator of invasion (Hulme, 2006) and needs to be mitigated. Research targeting effective management practices is also of major importance but, above all, it's important that it reaches managers and is applied in the field, which frequently fails to happen (Hulme, 2006; Andreu, Vilá & Hulme, 2009). Another major issue in invasion biology, which is crucial in all stages of management, is the accurate identification of species as misidentifications can have very serious consequences.

Management of invasive species should integrate a sequence of "key steps", some of them more cost-effective and promising in terms of achieving lasting and successful results than others, including: (1) prevention; (2) early detection and rapid response; (3) eradication; (4) containment; (5) control; (6) restoration and mitigation; (7) monitoring and evaluation (Wittenberg & Cock, 2005; Hulme, 2006; Pyšek & Richardson, 2010). Several of these steps are sequential, but not necessarily all, as some of them may be simultaneously applied (*e.g.* sequential steps with the subsequent taking place when the previous fails 1 → 2 → 3 → 4 → 5 → 6 → 7 as opposed to simultaneous application of several options 1 → 2 → 3 / 4 / 5 / 6 → 7) (Figure 1.5).

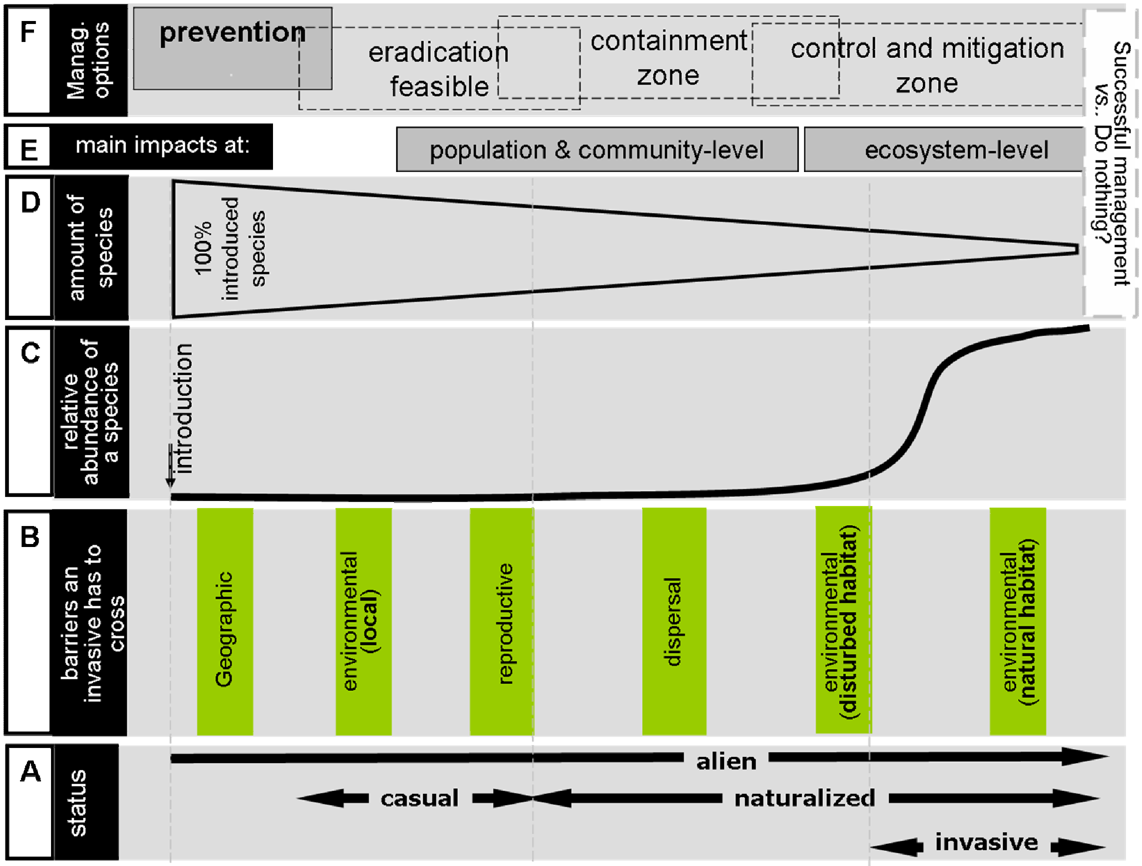


Figure 1. 5 Introduction-invasion continuum (A, C). Amongst all the species introduced only a limited number (D) will cross several barriers (B) becoming invasive; if a species establishes successfully it will have impacts (E); management actions (F) have to be set according to each invasion phase. Impacts (E) include even subtle changes that result from the species entering the system but that are not always easily perceived. The zones of management options (F) are approximate and some may overlap more than represented: *e.g.* prevention is crucial before introduction but may continue afterwards in order to prevent increased spread; eradication has higher probability of success prior to naturalization but can be achieved in the earliest phase of expansion; management options only apply to the species that are or have high potential to be invasive (based on Hulme (2006), Grice (2009) and Pyšek & Richardson (2010)).

Before embarking in expensive management options it is essential to prioritize the management actions, the species and the areas, taking into consideration factors such as the level of impacts, probabilities of success, value of ecosystem after recovery and available resources (Hulme, 2006). This is not straightforward and it is often additionally complicated by the existence of species in distinct stages of invasion, requiring distinct management options to be applied at the same time (Hulme, 2006; Pyšek & Richardson, 2010). Above all, there are no miraculous solutions and the easy options are rare. To achieve suitable levels of success, persistence and, as far as is possible, scientific-based decisions are essential.

Prevention

Preventing the introduction of species with high risk of becoming invasive is one of the most cost-effective management strategies (Pyšek & Richardson, 2010). Several actions are considered in prevention, such as risk assessment, border interception, pathway and vector management, legislative frameworks and public awareness. Some of these options are discussed below; public awareness is discussed afterwards due to its crucial and transverse role in several other management options.

Risk assessment of an alien species potentially invasive is the determination of the likelihood that the species might be introduced into a region (intentionally or accidentally), establish itself and become invasive (Figure 5) with considerable undesired impacts (Stohlgren & Jarnevich, 2009; Pyšek & Richardson, 2010). Basically, risk assessment procedures involves information gathering which aims to characterize the invasiveness of the species, the invasibility of habitats of introduction, the potential distribution and undesired effects to the environment, human-health and economy (Stohlgren & Schnase, 2006). If the risk assessment scores a species as representing high risk then action should be put in place to prevent that species from entering/spreading in the country/region. With this aim, risk assessment have been mainly applied pre-border, although it may also be applied afterwards, *e.g.* to develop invasion risk maps (Chytrý *et al.*, 2009) or to prioritize invasive species (Stohlgren & Jarnevich, 2009). Risk assessment is also one of the procedures frequently needed to support the decision of granting permission to release an exotic biological control agent (EPPO, 2009).

Increasing availability of databases of introduced species (*e.g.* Global Invasive Species Database, http://www.issg.org/database/welcome/; Rod Randall's Big Weed List, http://www.invasive.org/gist/biglist.html) has contributed to increasing accuracy rates of screening procedures. Some of them, *e.g.* Australian weed risk assessment (WRA), may be applied almost universally (within taxonomic groups), but with some modification needed (Gordon *et al.*, 2008; 2010; McClay *et al.*, 2010). Previous history of invasion elsewhere is one of the fundamental components of risk assessment, although screening procedures can identify high risk species which have no previous invasive record (Pyšek & Richardson, 2010).

Pathway and vector management is one of the key mechanisms for reducing unwanted introductions (Pyšek & Richardson, 2010). Identifying the pathways and vectors of introduction and dissemination of alien species allows adoption of proactive measures (*e.g.* targeting specific pathways to be monitored) that will reduce propagule pressure and consequently the likelihood of establishment and spread. For instance, one of the major pathways of dispersal for plants is commercial trade in ornamental species. Realising this focuses public awareness on finding alternatives for ornamental invasive plant species and development of legal frameworks to limit introductions. Nevertheless, pathways are numerous and the enormous volume of trade in commodities makes the goal of intercepting all the potentially problematic species almost impossible (Pyšek & Richardson, 2010). With this in mind it is easy to understand that additional management options are needed for the species that successfully cross this “stage”.

Early detection and rapid response (EDRR)

For species that are introduced or keep actively spreading, the most effective way of minimizing impacts is to detect new introductions, small invasions or spreading propagules of species already spread as early as possible, when populations are still localized, and quickly put in place procedures to eradicate, contain or control them (Holcombe & Stohlgren, 2009). Considering the expansive range of organisms that can be introduced and are potentially invasive (from microrganisms to large plants and animals), the difficulties of accurate taxonomic identification, the difficulty of detecting individuals in low numbers and the vast areas that can/need to be monitored, the probabilities of early detection of an invasive species are very low (Hulme, 2006; Gordon *et al.*, 2010; Pyšek & Richardson, 2010). Nevertheless, EDRR is highly cost-effective, justifying major research and management efforts to improve protocols and techniques. These processes include, for instance, (1) use of remote sensing to monitor and map species, (2) modelling potential distribution maps to target areas with higher probability of incursions, and (3) development of user-friendly identification guides and high-tech diagnostic tool (*e.g.* DNA bar-coding) (Pyšek & Richardson, 2010).

Early detection should set priorities, regarding species (*e.g.* watch lists) and areas (*e.g.* habitats with higher conservation value or areas that are more prone to invasion) in order to increase efficiency. Small invasion foci should be targeted first as they have increased invasion potential, because that will more efficiently slow the spread than if expansive populations were targeted (Rejmánek & Pitcairn, 2002). Rapid response should start with a quick inventory of the populations which is essential to avoid missing isolated patches or individuals that can then nullify the possibility of eradication. Additionally, it is essential that the infrastructure is in place, including control strategies, monitoring schemes, funding and human resources. Public awareness, including species identification skills, can be a major ally (Holcombe & Stohlgren, 2009).

Eradication

Eradication is the elimination of all the individuals of a species (including the seed-bank) within the management unit (*e.g.* country, conservation area) (Parkes & Panetta, 2009). It is more commonly applied to species with limited ranges and is a cost-effective option because, if successful, it precludes negative impacts before they appear. Nevertheless, it is difficult to implement because managers/ stakeholders frequently cannot be persuaded to act upon species that are not yet a problem in their lands/areas (Parkes & Panetta, 2009; Pyšek & Richardson, 2010).

Eradication should only be attempted if it is considered to be feasible to avoid failure and associated wasted effort and financial costs. To be considered feasible, it should ideally meet three criteria: (1) the average annual rate of increase of the population should be lower than the average annual long-term removal rate, not forgetting that funding has to be guaranteed; (2) there is not a source of new propagules; and (3) there are not adverse effects of the removal of the species (*e.g.* increase of other exotic species) that outweigh the benefits of removing the target plant (Parkes & Panetta, 2009). The potential impacts of the species if not eradicated should also be included in the analysis of feasibility. Regarding the feasibility of eradication, experiments showed that areas < 1 ha are likely to be eradicated, areas of 1-100 ha are expected to succeed in only about 33% of attempted areas and for larger areas expected success is low (Rejmánek & Pitcairn, 2002). Commonly, costs of invasive plants eradication increase exponentially as the area of invasion expands (Parkes & Panetta, 2009).

Once eradication is no longer feasible, containment and control are the most obvious options; both should be planned and developed as long-term strategies, with special care given to guaranteeing resources and continued participation of the various stakeholders (Grice, 2009). In general, the earlier the operations commence the higher is the likelihood of them being successful and the more cost-effective they become. Prioritization of species and places should be defined but this may be complicated by conflict of interests amongst stakeholders. Several scenarios are possible, from directing efforts to regions with lower abundance of the species (because this is a more cost-effective option that will avoid, or at least delay, impacts from getting worse) to directing efforts toward places with higher abundance of the species, which are already highly degraded, aiming to prevent spread into surrounding areas. Another alternative is to focus efforts on areas of particular interest (*e.g.* conservation) irrespective of the abundance of the invader or the costs. In such areas a multiple invasive species management approach can be considered (Grice, 2009).

Containment

Containment aims to limit the spread of an invasive species through management strategies applied to the periphery of its range, in order to prevent its range from reaching its full potential (Kriticos, Alexander & Kolometz, 2006). Natural barriers to range expansion (*e.g.* mountains, rivers,…) can and should be exploited. Containment can be considered partial if, instead of limiting spread entirely, it attempts to slow the rate of spread (Cacho, 2004). In either case, it can be applied to distinct management units (*e.g.* country, conservation area) as long as the species is expanding its range and curtailing its expansion is still a possibility (Grice, 2009). When the area occupied by a species is very large and composed of distinct smaller units, the periphery gets larger and the option to control becomes more realistic than to contain. Containment is more appropriate to species that disperse slowly and over short-distances and for which effective barriers can be set in place (Hulme, 2006). A containment programme should include a series of activities such as 1) accurate identification of the species, 2) establishment of a scheme to detect the target species outside the range where it is being contained, 3) planned subsequent activities to remove it if detected, and 4) adequate policies and proper implementation of species management (Grice, 2009).

Control

Control activities aim to reduce the impact and the abundance of an invasive species to an acceptable level in the long-term but, unlike containment, not necessarily limit its range (Hulme, 2006; Grice, 2009). The balance between perceived or predicted impacts as opposed to predicted costs of control, the stage of invasion and the availability of effective control measures determine whether control is the best option (Grice, 2009). Predicting the impact of a species is also a fundamental, though difficult, aspect in making the decision for control (as opposed to eradicate or contain). Incomplete knowledge (*e.g.* predicting impacts) should not delay the process as an early response can be critical in achieving success. To achieve a satisfactory level of control, the operation should include three levels: 1) initial control, which aims at drastic reduction of the invasive target species, which is usually very costly; 2) follow-up control, aiming to reduce any reinvasion after the initial control, which can include seedlings, root suckers and coppice; and 3) maintenance control to sustain the invader at reduced levels, usually at lower costs and at long term (Campbell, 1993). In the case of plants, knowledge of seed longevity, germination requirements, capacity for vegetative reproduction and time to first reproduction determines how long the cleared area should be monitored to set follow-up (and latter, maintenance) control in place (Grice, 2009).

For eradication, control and containment, methods applied include mechanical, chemical, and biological control, habitat management and integrated management. In the context of this study, the focus will be on biological control (see section below).

Restoration and Mitigation

Mitigation, in its strict sense, focuses on the affected native species rather than in the invasive species (Wittenberg & Cock, 2001). The interventions of restoration and mitigation range from the simple removal of an invasive species to a variety of options that aims to favour natives, or even more complex options that involve engineering, native species reintroduction or translocation of a viable population of the endangered species to a "healthy", non-invaded ecosystem (Wittenberg & Cock, 2001). Efforts are often complicated by interacting impacts which may create novel functions within the system resulting in unpredictable reactions after control (*e.g.* secondary invasions, elevated levels of nitrogen following invasion that will remain after control and preclude natives, natives being damaged by control because had became reliant on the invasive species) (Pyšek & Richardson, 2010). All these issues have to be considered cautiously when planning interventions in order to avoid, or at least decrease, unwanted effects.

"Do nothing" as an option

When resources are scarce or no effective control measures are available, or when the degradation is already so profound that chances of successful recovery are very low or even nonexistent (see Box 1.1), doing nothing can be the best option, at least until some of the above scenarios change. This approach will avoid wasting resources that can be most effectively used into other areas or management actions. "Do nothing" about invasive species management, *i.e.* choosing to "live with the problem", does not mean that mitigation actions should not take place.

Box 1.1 "Novel ecosystems" instead

Several authors advocate that in many ecosystems, but particularly in the ones where human intervention is particularly high, transformations are such that restoration of invaded habitats aiming to recover the original system in pristine condition is not a realistic or even possible goal (Hobbs *et al.*, 2006; Richardson *et al.*, 2007; Hobbs, Higgs & Harris, 2009; Pyšek & Richardson, 2010). In such drastically changed ecosystems the difficulty of removing the invaders may require a more pragmatic analysis with invaders considered as species that change their ranges and the communities into which they are introduced, and may even be needed to guarantee some ecosystems services (Walther *et al.*, 2009). As such, it is almost inevitable that new ecosystems will have to be developed as "novel ecosystems" or "emerging ecosystems". The possible outcomes of restoration may be hybrid systems with some of the characteristics of the previous system and other novel elements, or novel systems, with different species, interactions and functions (Hobbs, Higgs & Harris, 2009)

Monitoring and evaluation

Feedback from results of management actions is needed in order to modify, or even abandon, ineffective strategies, to allow other managers/stakeholders to learn from experience and even to validate the management programme. These issues can only be achieved through monitoring and evaluation of the actions taking place; without them the programme is not complete. Evaluation of actions should include information about the population of the target invader(s), the condition of the area under management, and changes in species composition. Establishment of specific targets at the beginning of the management programme will help to more-accurately evaluate its success or failure afterwards (Wittenberg & Cock, 2001).

Public Awareness and Education

Public awareness and education are often considered as an important part of prevention but they should also be seen as essential to other management options (Wittenberg & Cock, 2001). Activities should target technicians, stakeholders and the public in general who are important vectors of introduction and spread of invasive species (Ruiz & Carlton, 2003). A well informed public will adopt a more responsible attitude (*e.g.* selecting native or non-problematic exotic species; being aware of introduction pathways and excluding them; adopting measures to avoid being an "accidental vector" of seeds) and become active (*e.g.* contributing to early detection programmes; controlling species in private lands) with significant repercussions for species management. Awareness activities should deal with issues such as environmental and economic risks involved with IAS, laws and regulations to prevent introductions of alien species (*e.g.* reasons for the restrictions; regulatory actions), species recognition and easy, practical things to do in order to collaborate with IAS management. Printed materials, video presentations, talks, workshops, interactive games and hands on activities are all promising approaches (Wittenberg & Cock, 2001; Marchante *et al.*, 2010).

Biological control: the most sustainable option?

Biological control (= biocontrol) consists in the use of a species (host-specific) to control another species that has become problematic; the first species is considered to be a natural enemy of the second. According to Pyšek and Richardson (2010) biocontrol "has become and will remain the foundation of sustainable control efforts for many invasive species, especially plants, in many regions". Several other authors recognized it as the most "environmentally friendly" and cost effective management practice (Holden, Fowler & Schroeder, 1992; Murphy & Evans, 2009; Wilson *et al.*, submitted). Biological control includes several different techniques with classical biological control (CBC) being the most commonly used against invasive species. CBC is based on the Enemy Release Hypothesis (Keane & Crawley, 2002) (see above) and when applied to plants searches for host-specific herbivores or pathogens in their native range and afterwards introduces them into the new exotic location, where the plant has become problematic (Harley & Forno, 1992). The aim is to control exotic invasive species in an attempt to "rebalance nature", *i.e.*, remove the competitive advantage that the species had acquired when it was initially transferred, and becoming equivalent to the native species which has their own natural enemies. The aim of CBC is not to eradicate the invasive species but instead to reduce it to levels where it no longer causes a significant problem (Hulme, 2006) though supporting survival of the agent itself and allowing self-replicating populations to persist without the need for additional efforts of reintroductions.

Biological control has been used for over a century worldwide, with the first international transfers in the 1870s. Initially it was mostly used against plants and insects that caused problems to agriculture crops, but more recently it has begun to be used also against invasive species, mainly plants and arthropods (Murphy & Evans, 2009). Nevertheless, the extended use against invasive species was not adopted in Europe (Sheppard, Shaw & Sforza, 2006) until early 2010, when the first official release occurred in the UK (Djeddour & Shaw, 2010). Worldwide, nearly 400 agents have been released against over 130 plant species in 70 countries (Julien & Griffiths, 1998). Many introductions of biocontrol agents resulted in either complete or moderate levels of success. Complete biocontrol has been roughly estimated for ca. 30-39% of total cases of invasive plants, while estimations are that a higher number of agents have contributed significantly to control de target (Murphy & Evans, 2009). The earliest successful examples included control of the New World prickly pears (*Opuntia* spp.) in Australia, in the 1920s, using the moth *Cactoblastis cactorum*. Nevertheless, failures either due to failing to achieve successful control or due to non-target effects are also common. The records of biocontrol agents who have become problematic through direct, and less frequently indirect, non-target effects (Louda *et al.*, 2003; Louda & Stiling, 2004; Messing & Wright, 2006) came mainly from the first half of 20th century, when tests performed pre-release were less rigorous and risks were not considered in the same way as presently. The problematic cases pushed biological control to become a highly specialized science, whose protocols address maximizing both safety and success and thereby minimizing ecological risks (Murphy & Evans, 2009). The practice of selecting efficacious agents has progressed but is not yet "satisfactory". Assessing ecological risks prior to release is also not entirely possible (particularly regarding indirect effects) but host specificity screening can accurately determine the feeding characteristics of agents. Presently most host specificity testing is based on a centrifugal phylogenetic method (Briese, 2002). Unfortunately, too much attention has been paid to the few cases where there have been non-target effects (mostly from agents that had proved to be non-specific during risk assessment) and not enough on the many successful cases (mostly ignored because the target pests are no longer a problem) and this lead to public, even within scientific fora, apprehension about CBC of invasive species (Murphy & Evans, 2009). Despite CBC can be considered as potentially dangerous, current practice assures that before a species is cleared for release a full range of tests are performed in order to assure that the risk is minimal. In fact, CBC is seen by many as the only safe, practical and economically feasible method to control invasive species that is sustainable in the long term (Murphy & Evans, 2009).

THE STUDY ORGANISM: *ACACIA LONGIFOLIA*

The genus *Acacia sensu lato* (*Leguminosae, Mimosoideae*) is one of the largest plant genus comprising over 1380 species, mainly native to Australia and Pacific (993 species), Americas (185 species) and Africa (144 species) (Maslin, 2001) (Figure 1.6). Beyond their native range, acacias have been extensively introduced all around the world and large stands are nowadays very frequent, both under cultivation and as invasives. Being so numerous and widely used and problematic as invaders, publications about the genus are plentiful (*e.g.* Jones, Roux & Warren (1963); Milton & Moll (1982); Holmes & Cowling (1997); French & Major (2001); Hoffmann *et al.* (2002); Walters & Milton (2003); Impson, Moran & Hoffman (2004); Yelenik, Stock & Richardson (2004); Forrester, Bauhus & Cowie (2005); Hagos & Smit (2005); Rodríguez-Echeverría, Crisóstomo & Freitas (2007); Wood & Morris (2007); Marchante *et al.* (2009); Hellmann *et al.* (2010); Smith (2010)). Particular interest in the Australian acacias prompted an international workshop, “Human mediated introductions of Australian *Acacia* species: a global experiment in biogeography” whose results will be published in a special issue of the journal *Diversity and Distributions* in 2011.

Acacia sensu lato is now known to be a polyphyletic group (Murphy, 2008) (subgenus *Acacia* is not closely related to subgenera *Phyllodineae* and *Aculeiferum*) and as such the maintenance of the genus *Acacia* as it was formerly known has been subject to much recent discussion and may be split in the future.

Figure 1. 6 Indicative native range of the genus *Acacia sensu lato* [source: World Wide wattle http://www.worldwidewattle.com/infogallery/distribution.php#australian].

In fact, in the XVII International Botanical Congress (IBC) in Vienna (2005) the retypification was ratified with the acceptance of a new type for *Acacia*, namely *A. penninervis* as a replacement of the African/Asian species, *A. nilotica*. Implications are that when, and if, the genus *Acacia sensu lato* is divided the name *Acacia* will apply only to the ca. 1000 species, mostly Australian, now belonging to the subgenus *Phyllodineae*. If so, most of the species from other subgenera will become known as either *Vachellia* or *Senegalia* (Table 1.4). Nevertheless, the changes (including both retypification and alternatives to new names) are contentious both amongst the international botanical community (Moore *et al.*, 2010) and the general public (Carruthers & Robin, 2010) and there are chances that the IBC 2005 decision may be reversed (Maslin, 2008).

Table 1. 4 Alternatives of generic and subgeneric names for *Acacia sensu lato* and numbers of accepted species (source: World Wide Wattle, http://www.worldwidewattle.com/).

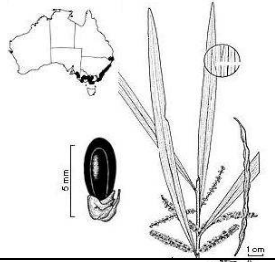
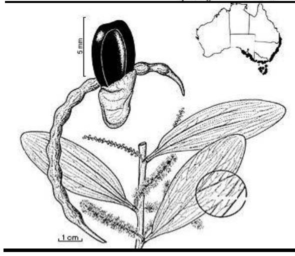
Pre-IBC names (A. nilotica – type)	***Post-IBC names (*A. penninervis *- type);*** Acacia sensu lato *treated as:*		***No. of species***	***Indicative distribution***
	a single genus	**multiple genera**		
Subgenus *Acacia*	*Subgenus 'nilotica group'*	*Vachellia*	c. **163**	Americas, Africa, Asia, Australia
Subgenus *Aculeiferum*	Subgenus *Aculeiferum*	*Senegalia*	**203**	Americas, Africa, Asia, Australia
Section *Filicinae*	Section *Spiciflorae*	*Acaciella*	**15**	From south-central U.S.A. to Argentina
'*Acacia coulteri*' group	Section *Filicinae*	*Mariosousa*	**13**	From Arizona to Costa Rica
Subgenus *Phyllodineae*	Subgenus *Acacia*	*Acacia*	**987**	Australia (+); Pacific region (to Hawaii and Taiwan), Madagascar and Mascarene
Total number of species			**1381**	

Acacia longifolia (Andrews) Willd. (Sydney golden wattle, also named long-leafed wattle) belongs to the (former) Subgenus *Phyllodineae*. It is a small nitrogen-fixing tree or shrub (Rodríguez-Echeverría, Crisóstomo & Freitas, 2007), 1.5–10 m tall, native to south-eastern Australia (Paiva, 1999), which is invasive in Portugal, particularly in dune ecosystems (Marchante, 2001; Marchante, Marchante & Freitas, 2003), and South Africa (Dennill *et al.*, 1999), among other regions (Elorza, Sánchez & Vesperinas, 2004; Kutiel, Cohen & Shoshany, 2004). It has linear to elliptic phyllodes, 5–25 cm long x 10–35 mm wide, with 2–4 prominent primary veins; the inflorescences form spikes, solitary or twinned, 2–5 cm long (Figure 1.7); the pods are cylindrical or subcylindrical, 5–15 cm long x 4–10 mm wide and can be straight to curved; seeds are elliptic, sometimes irregularly shaped, 4–6 mm long, shiny; funicle folded several times into a thickened lateral skirt-like aril (Maslin, 2001).

Figure 1. 7 *Acacia longifolia*: a. branch with small flower buds, b. inflorescences forming spikes, c. cylindrical pods that can be straight to curved, d. seed with funicle folded several times.

Some authors (Whibley, 1980) recognise two subspecies within this taxon (Table 1.5): *A. longifolia* (Andrews) Willd. subsp. *longifolia* and *A. longifolia* subsp. *sophorae* (Labill.) Court., while others treat *A. longifolia* and *A. sophorae* as distinct species (Paiva, 1999).

Table 1. 5 Distinctive morphological characteristics, including illustrations (by K. Thiele), of *A. longifolia* subsp. *longifolia* and *A. longifolia* subsp. *sophorae* (adapted from World Wide Wattle http://www.worldwidewattle.com /speciesgallery/longifolia.php?id=17861).

	*A. longifolia* = *A. longifolia* subsp. *longifolia*	Erect or spreading shrub or tree 1-8 m high. Phyllodes 6-20 cm long, mostly 4-20 mm wide, 2 or 3 or more longitudinal veins more prominent, apex usually acute or subacute. Pods straight to curved, sometimes curled back or twisted on opening.
	*A. sophorae* = *A. longifolia* subsp. *sophorae*	Prostrate or decumbent shrub 0.5-3 m high, sometimes taller. Phyllodes 4-11 cm long, 10-30 mm wide, 2-4 longitudinal veins more prominent, apex subacute or obtuse. Pods often coiled and twisted on opening.

Both taxa show some morphological differences that in some situations seem to be ecological adaptations to the different environments where they occur (*A. longifolia* subsp. *sophorae* usually grows closer to ocean and subsp. *longifolia* is typically more inland), which seems to be the case in Portugal (H. Marchante, *unpublished data*). *Acacia floribunda* (Vent.) Willd is also closely related to *A. longifolia* and has earlier been considered as another subspecies (Maslin, 2001).

WHY DUNE ECOSYSTEMS?

Coastal dune ecosystems are considered a vulnerable ecosystem by IUCN and should have the highest priority for conservation action, namely when significant biodiversity values are at risk (Shine *et al.*, 2000). They are not only fundamental barriers against the advance of the ocean but also harbour many plant species exclusive to dune systems, several of them endemic, and form unique habitats which confer them high ecological value (Carter, 1995; Honrado *et al.*, 2006). The stability and natural dynamics of coastal dunes relies on natural plant communities which bind the labile sands and minimize the effects of erosion (van der Putten & Peters, 1995).

Coastal ecosystems are considered amongst the ecosystems most seriously threatened by IAS, including in the Mediterranean (Chytrý *et al.*, 2009; Pyšek & Richardson, 2010). In many regions of the world, e.g., Australia (Mason, French & Russell, 2007), California (Beckstead & Parker, 2003), NW European countries (Kollmann *et al.*, 2007), Korea (Kim, 2005), Israel (Kutiel, Cohen & Shoshany, 2004), Portugal (Alves *et al.*, 1998; Marchante, Marchante & Freitas, 2003; Rodríguez-Echeverría, Crisóstomo & Freitas, 2007; Marchante *et al.*, 2008b, a) and Spain (Sobrino *et al.*, 2002), studies with different perspectives show that invasive plant species modify, or have the potential to affect, coastal ecosystems in many different ways, including degradation of biodiversity. Moreover, the invaders frequently belong to life forms previously underrepresented in several habitats of dune ecosystems, thereby causing major transformations of the vegetation structure (Mack, 2003; Marchante, Marchante & Freitas, 2003) and even loss of habitat diversity.

Native coastal sand dunes in Portugal are characterized by communities that extend from the ocean to the interior ordered into a spatial succession of plant associations (each with its own species assemblage), that typically replace themselves after disturbance, and conceal remarkable floristic and phytocoenotic diversity (Neto, 1993; Honrado *et al.*, 2006; Silva, 2006). The dune species are naturally adapted to nutrient poor sands, drought, plenty of light at soil level (Smith & Huston, 1989) and, depending on the distance to the ocean, sand shifting and salinity. Natural coastal dune ecosystems of northern Portugal are generally composed, from the ocean to inland (Figure 1.8) of: (1) a first zone of bare sand, known as the embryonic dunes, occasionally washed by the sea during storms and with sporadic small plants; (2) a second zone with shifting sands, composed of elevated dunes, normally parallel to the shoreline, where perennial herbaceous species dominate, known as the primary dune or foredune; (3) a more depressed zone known as interdune, where sands are stabilized and dominated by shrub and sub-shrubs species comprising a few small trees occurring infrequently; in the interdunal areas commonly occur sandy depressions with freshwater wetland communities; (4) another elevation (secondary dune) also dominated by herbaceous species. The number of interdune (depressions) /secondary dune (elevations) sequences that occur in the interior dunes may be variable and depends, e.g., on the extension of the dune system, wind regimes, topography and occurrence of obstacles to movement of sands (Alves *et al.*, 1998). Beyond the interior dunes, there is frequently another zone with sands already consolidated allowing, together with dune species, the occurrence of other species such as *Pinus* spp. and *Juniperus* spp. There is frequent overlap and local variation in the zones which frequently makes them difficult to distinguish in the field.

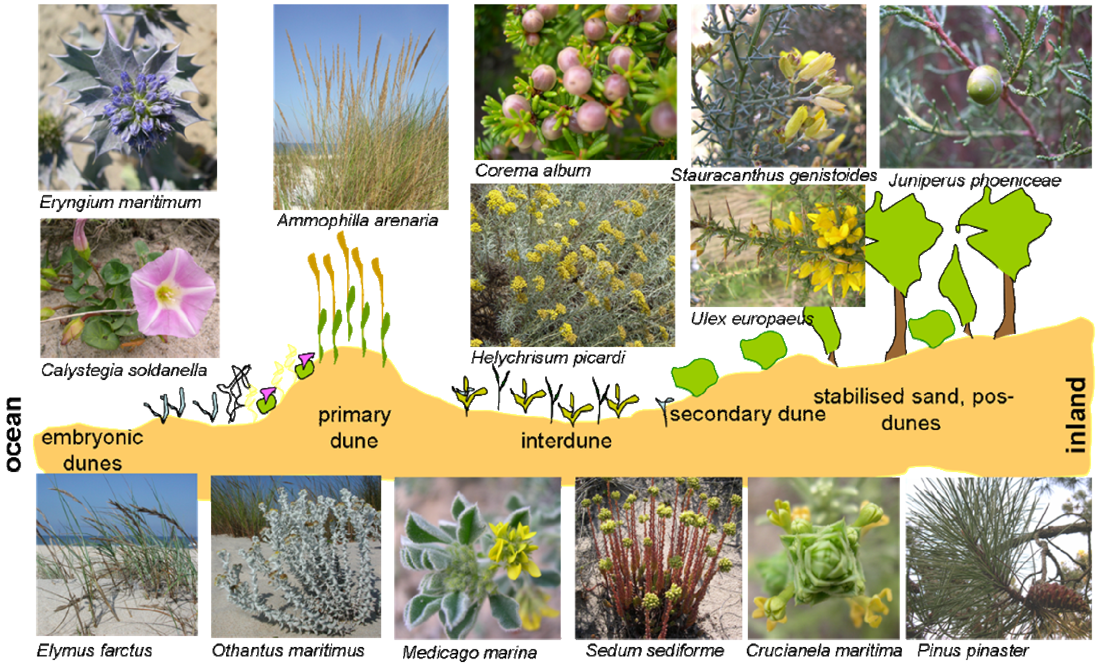


Figure 1. 8 Representation of the typical dune system of the coastal ecosystems of northern Portugal with some of the characteristic plant species. All the species represented occur in the study system (São Jacinto Dunes Nature Reserve, see below).

Along the Portuguese coast, pristine dune systems with all the zones populated by their respective typical plant communities are becoming increasingly rare due to several factors, namely forestry plantations, exploitation for agriculture, tourism, sand extraction, construction and invasion by alien species (Rei, 1924; Alves *et al.*, 1998; Marchante, Marchante & Freitas, 2003; Silva, 2006; Marchante *et al.*, 2008b). This last threat, in particular, is explored in the present study. Numerous native species in dune ecosystems are being replaced by invasive species which include *A. longifolia* (Alves *et al.*, 1998; Marchante, Marchante & Freitas, 2003), *Acacia saligna* (Labill.) H.L. Wendl. (Marchante, 2001), *Carpobrotus edulis* (Campelo, 2000) and *Cortaderia selloana* (Marchante, Freitas & Marchante, 2008).

Acacia longifolia, the most prominent and widespread invader in Portuguese dunes, was introduced over 150 years ago to curb sand erosion. Besides deliberate planting, its abundance and distribution has increased greatly following fire events (Marchante, Marchante & Freitas, 2003). One of the Portuguese coastal dune ecosystems where *A. longifolia* has become more widespread and problematic includes São Jacinto Dunes Nature Reserve, with high value for conservation (Decreto Regulamentar nº 24/04, de 12 de Julho).

São Jacinto Dunes Nature Reserve

São Jacinto Dunes Nature Reserve (SJDNR) is located at the central-northern coast of Portugal (40º 39' N, 8º 44' W). It was created in 1979 (Decreto-Lei nº 41/79, de 6 de Março) and reclassified in 1997 (Decreto Regulamentar nº 46/97, de 17 de Novembro). Later in 1999 it was proclaimed the Special Protection Area (SPA) "Ria de Aveiro" to conserve wild bird species (Decreto-Lei nº 384-B/99, de 23 de Setembro), which integrated Natura 2000 Network, and included SJDNR. Finally in 2004 (Decreto Regulamentar nº 24/04, de 12 de Julho) SJDNR was reclassified again maintaining the protection status and extending the limits of the reserve from 660 ha to 960 ha.

The climate of SJDNR is Mediterranean with an Atlantic influence. Dominant winds blow from Northwest (NW) and North (N), approaching from the Ocean (additional climatic details are given in chapters 2, 3, 4 and 5). The soils are arenosols according to FAO classification (Rogado *et al.*, 1993).

Perennial plant communities observed in SJDNR include two communities on mobile dunes (one on embryonic and other on primary dunes) and several communities on interior dunes (interdune and secondary dunes), including chamaephytic communities of perennial acidophilous grasslands and dwarf shrublands that coexist in a mosaic (Table 1.6) (Silva, 2006).

Several endemic species, including Portuguese, Iberian and European endemism, are present (*e.g. Antirrhinum majus* L. subsp. *cirrhigerum* (Ficalho) Franco*, Corema album* (L.) D. Don*, Iberis* *procumbens* Lange subsp. *procumbens, Juniperus phoenicea* L. subsp. *phoenicea, Stauracanthus genistoides* (Brot.) Samp.*, Tuberaria guttata* (L.) Fourr.*, Ulex europaeus* L. subsp. *latebracteatus* (Mariz) Rothm. and *Vulpia alopecuros* (Schousboe) Dumort.) (Figure 1.8) which shows the importance of the communities present in SJDNR. Several zones characteristic of a pristine dune system, with their typical communities, are still present in some areas of the SJDNR although their conservation status is highly variable and hardly ever incorporating the complete sequence (from embryonic to interior dunes "in a row") in pristine condition. Nowadays they are very frequently overwhelmed by exotic invasive species (Figure 1.9).

Table 1. 6 Typical zones of the dune ecosystems of the northern Portuguese coast, respective perennial plant communities and the most representative species occurring in SJDNR (base on Alves *et al.* (1998); Honrado *et al.* (2006) and Silva (2006).

INLAND -- OCEAN

Dune zone		Plant Communities / associations	Some of the plant species more common
Mobile dunes	Embryonic dunes	*Euphorbio paraliae-elytrigietum boreoatlanticae*	*Calystegia soldanella, Elymus farctus* subsp. *boreo-atlanticus, Eryngium maritimum, Euphorbia paralias*
	Primary dunes	*Otantho maritimi-ammophiletum australis*	*Aetheorhiza bulbosa* subsp. *bulbosa, Ammophila arenaria* subsp. *arundinacea, Artemisia campestris* subsp. *maritima*, *C. soldanella, E. maritimum*, *E. paralias*, *Otanthus maritimus*, *Medicago marina*, *Pancratium maritimum*
Interior dunes (Interdune and Secondary dune)	Chamaephytic communities	*Iberidetum procumbentis sedetosum sediformis* *	*Antirrhinum majus* subsp. *cirrhigerum, A.campestris* subsp. *marítima, Corema album, Crucianella maritima, Helichrysum italicum* subsp. *picardi, Malcomia littorea, Medicago marina, P. maritimum, , Sedum sediforme, Seseli tortuosum, Vulpia alopecuros*
	Acidophilous grasslands	*Sedo sediformis-corynephoretum maritimi* *	*Corynephorus canescens* var. *maritimus, S. sediforme,V. alopecuros*
	Dwarf shrublands	*Stauracantho genistoidis-corematetum albi* *	*Cistus salviifolius, C. album, H. italicum* subsp. *picardi, Stauracanthus genistoides, Ulex europaeus* subsp. *latebracteatus*

* Communities endemic to the Mediterranean coastal-lusitanean dunes.

Late in the 19th century, early in the 20th century along the Portuguese northern coast, including in SJDNR, efforts aimed at dune stabilization were very frequent and consisted of installation of palisades (at least in some of the locations), followed by plantation of *Pinus pinaster* Aiton and *Myrica faya* Aiton and afterwards sowing of several exotic species, namely *Acacia* (mainly

A. longifolia but also other species that did not become so widespread), and *Carpobrotus edulis* (Neto, 1993); subsequently, existing shrub species (*Cytisus* spp., *Ulex* spp.,) were promoted in several locations of the coast aiming to increase cover of the bare sands (Rei, 1924). In SJDNR, *A. longifolia* proliferated after the initial introduction, and several fire events over time triggered its exponential growth, resulting in the large monospecific stands that are currently common.

Figure 1. 9 Contrast between the native dune communities (images on the left) *vs.* the invaded areas (images on the right) in SJDNR.

When this work was carried out, *A. longifolia* constituted a near-monoculture over 150 ha of SJDNR and occurred at lower densities in association with other species over 200 ha (Guimarães, 2004), extending from the foredune to the zone of consolidated sands.

THESIS OUTLINE AND OBJECTIVES

This work, through its five main chapters, aims to contribute to fill some gaps in invasion *Ecology,* and additionally to contribute with practical management options that will be useful to managers.

The first aim of this thesis – chapter 2 - was to evaluate the effect of the invasive *Acacia longifolia* at vegetation level, in areas with different invasion durations (stands long-invaded for more than 20 years, *vs.* stands recently invaded occupied after a summer fire in 1995) over a five years period. This allowed, simultaneously and in addition, addressing two issues seldom studied: quantification of impacts in the long-term and effects of duration of invasion.

The anticipated key role of the invader seeds in perpetuating the invasion leaded to the second aim - chapter 3: to increase knowledge about the seed ecology of *A. longifolia,* namely quantifying the seed rain, assessing seed viability before and after entering the soil and measuring the accumulated soil seed bank.

Ideally, before embarking in expensive management plans of an invasive plant the likelihood that restoration efforts will succeed should be assessed (Zavaleta, Hobbs & Mooney, 2001). Therefore, the next question that this thesis intended to answer was whether the system still was resilient, *i.e.*, capable of autogenic recover after clearing. With this aim, two distinct approaches were considered:

- first (chapter 4), the soil seed bank was evaluated to test the potential resilience of the system and to access the validity of seedling emergency method to do it quickly; cleared *vs.* uncleared plots were compared;

- second (chapter 5), in experimentally cleared plots (considering *A. longifolia* removal with and without the underlying litter layer) the recovery of vegetation was monitored through time, from 2002 to 2008 achieving a recovery period longer than most post-clearing studies.

Both approaches (chapters 4 and 5) considered clearing of long- and recently invaded stands.

Because the methodologies available in Portugal for control of *A. longifolia* are expensive and do not produce satisfactory long-term results, a biocontrol agent successfully used in South Africa for more than 20 years was evaluated. The safety of using the Australian gall forming wasp (*Trichilogaster acaciaelongifoliae*), that targets seed reduction but also curbs the vegetative growth, was tested to reduce the invasive success of *A. longifolia* in Portugal (chapter 6); its implications are discussed and an overlook of the legal procedures needed before release is presented.

General introduction (chapter 1) and discussion (chapter 7) are included aiming to integrate the five main chapters (chapters 2-6) and trying to avoid much overlapping with each separate chapter.

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Part II

Chapter 2

Long term changes in vegetation structure of a Mediterranean coastal dune invaded for different durations by *Acacia longifolia*

ABSTRACT

Invasive alien species are one of the main threats to biodiversity worldwide, being considered a major threat to endangered species as well as to the integrity and functioning of ecosystems. Despite studies about impacts of invasive species are becoming very frequent, there is a scarcity of both long term studies and studies considering the effects of invasion time on native communities.

Diversity, species richness and cover of contrasting plant communities of a Mediterranean coastal dune were compared over a five-year period to assess the impacts of an invasive tree, *Acacia longifolia*. Three situations were compared, a recently invaded area, a long-invaded area and an area that had never been invaded. Plant traits, similarity between areas and species turnover were also analyzed because these measures potentially reveal patterns that are not apparent in comparisons of the single values derived from measures abovementioned.

Results showed that native species richness was lower in both recently and long-invaded areas than in uninvaded areas. As invasion time extended, species shared with native areas decreased, reductions in plant cover, evenness and diversity intensified and species turnover rates declined. Analyses of traits showed that: a) in both recently- and long-invaded areas geophytes, hemicryptophytes and chamaephytes were underrepresented while species of therophytes adapted to elevated nitrogen levels in the soil were overrepresented; b) in recently invaded areas, species typical of dunes were replaced by native and exotic generalists; c) in long-invaded areas terophytes and wind-dispersed species were underrepresented. The potential for reinvasion by *A. longifolia* was higher in long-invaded areas, while other exotic species were more frequent in recently invaded areas.

Synthesis. Dunes invaded by *A. longifolia* become transformed from almost treeless landscapes into highly modified woodlands which become more degraded the longer the invasion is left to progress. The invasion brings about a diversity of biotic changes in both the structure and function of the ecosystem which are yearly dynamic and apparently tend to stabilize after several decades. Analyses including only single-figures parameters conceal some of the changes taking place.

Keywords: dispersal syndromes, dominance diversity curves, IAS impacts, invasion *Ecology,* plant traits, Portugal, Raunkiær life forms, recently- *vs.* long-invaded areas, Sorensen similarity, turnover rates

INTRODUCTION

Invasive alien species (IAS) represent one of the main threats to biodiversity worldwide, being considered a major threat to rare and endangered species as well as to the integrity and function of ecosystems (Blossey, 1999; Millennium Ecosystem Assessment, 2005; Richardson *et al.*, 2008; Gaertner *et al.*, 2009). The impacts of IAS are numerous, severe and frequently irreversible, often intensifying with time (van Wilgen *et al.*, 2008; Lodge *et al.*, 2009). They affect biodiversity, decrease species richness and change community structure, species cover and environmental conditions (Gaertner *et al.*, 2009; Hejda, Pyšek & Jarosík, 2009; Jäger, Kowarik & Tye, 2009; Vilá *et al.*, 2010) while altering ecosystem functioning and disrupting ecological networks (Vitousek & Walker, 1989; D'Antonio & Kark, 2002; Ehrenfeld, 2004; Marchante *et al.*, 2008b; Heleno *et al.*, 2009; Bartomeus, Vilà & Steffan, 2010). Economic impacts, *e.g.* management actions and productivity losses (Lodge *et al.*, 2009), can be enormous and are usually more easily perceived by stakeholders. In Europe alone, costs have been estimated as ca. €10 billion annually; a conservative value as impacts of more than 90% of the IAS have not yet been quantified (Hulme *et al.*, 2009). Invasive plants are frequently spatially more localized than animals and have more ecological than economic impacts, making it difficult to attribute a financial value to their impacts (Vilá *et al.*, 2010). Regardless of their obvious impacts, sweeping generalizations such as IAS cause species extinction or that they rank 2nd as the most significant worldwide threat to biodiversity, should be avoided or at least rigorously contextualized when used (Sax & Gaines, 2008; Jäger, Kowarik & Tye, 2009).

Impacts of IAS are frequently assessed according to their influence on parameters such as species richness, diversity indexes or plant cover (Hejda & Pyšek, 2006; Hejda, Pyšek & Jarosík, 2009; Jäger, Kowarik & Tye, 2009) which, while useful, reduce changes in species assemblages to single values which over simplify the situation and thereby may obscure real patterns and trends. Species traits are characteristics that reveal species adaptations to the environment they occupy (Menezes, Baird & Soares, 2010) allowing complex clusters of species to be categorised into a relatively small number of groups. Both biological (*e.g.* life form, dispersal mode) and ecological (*e.g.* tolerance to nitrogen level, species biogeographic distribution) traits can be useful indicators of environmental change, *e.g.* invasion by exotic species, because they have the potential to reveal differences amongst species assemblages of distinct communities or alterations occurring in one community through time. This is possible because each species (with its own identity, adaptations and functions) is assigned a trait. Analyses of these species traits can augment patterns derived from single-value parameters, with no need for complex analyses of extensive species lists.

Coastal ecosystems are considered of high priority for conservation action by the International Union for Conservation of Nature (IUCN) (Shine, Williams & Gündling, 2000), being important ecological systems of native plants which retain the integrity of the coast line by promoting dune formation (Carter, 1995). Alien plant species have been frequently introduced to coastal dunes to curb sand erosion (Rei, 1924; Elorza, Sánchez & Vesperinas, 2004; Hulme *et al.*, 2008). With time, however, some of these same species have become a major threat to the ecosystems they were supposed to protect and dunes now rank among the most extensively transformed ecosystems due to invasive species (Beckstead & Parker, 2003; Marchante, Marchante & Freitas, 2003; Mason, French & Russell, 2007; Marchante *et al.*, 2008b). When coupled with increasing threats, such as sea levels rising, valuable dune ecosystems will probably become even more vulnerable to disruptive influences (Granek *et al.*, 2010).

Plant communities on Portuguese coastal dunes include a remarkable floristic and phytoceonotic diversity (Honrado *et al.*, 2006). Although the floristic assemblage does not include a high number of endemic species, several habitats (namely plant associations of the interior dunes) are considered unique (Honrado *et al.*, 2006; Silva, 2006) and a high priority for conservation. The invasion of these usually-open communities by IAS, including tree species, leads to drastic changes in the landscape (Alves *et al.*, 1998; Campelo, 2000; Marchante, Marchante & Freitas, 2003). *Acacia longifolia* (Andrews) Willd (Fabaceae) is one species which is invasive in several regions of the world (Henderson, 1998; Weber, 2003), including in Europe where it has proliferated on coastal dunes (Elorza, Sánchez & Vesperinas, 2004). Its impacts include declines in plant species richness and cover, changes in seasonal dynamic of plant communities (Marchante, 2001; Marchante, Marchante & Freitas, 2003; Hellmann *et al.*, 2010) and alteration of soil chemical and microbiological parameters with consequent changes to ecosystem functioning (Marchante, 2008; Marchante *et al.*, 2008b, a).

Considering the impacts measured so far, *A. longifolia* is a "transformer" (sensu Richardson *et al* (2000) or "ecosystem engineer" (see Conser & Connor (2009)). However, as for many other species and ecosystems, though important, median or long-term analyses of the transformation of plant communities through time are scarce and none seem to consider the influence of invasion duration (Strayer *et al.*, 2006; Carlsson *et al.*, 2010; Hellmann *et al.*, 2010). Considering that the dune systems which are particularly susceptible to invasion (Marchante, Marchante & Freitas, 2003) include many plant species with ephemeral life cycles (Silva, 2006) some of which persist as dormant seed banks (Marchante, Freitas & Hoffmann, 2010a) studying such systems for long time periods can reveal patterns that are easily overlooked when assessments are of short duration. This realisation provided the impetus for us to set up permanent plots in sand dunes that were either non-invaded, recently invaded and long-invaded by *A. longifolia.* The plots were monitored for 5 years aiming to assess the impact of *A. longifolia* on the composition of native plant communities and to measure species, traits and habitat changes over time. The reinvasion potential of *A. longifolia* and invasion by other exotic species were also analysed. We investigated the following questions: 1) Do impacts of invasion intensify as the invasion gets protracted? 2) Are patterns of impacts dynamic over a period of five years? 3) Can measurements of multiple parameters reveal impacts not noticed when single-value parameters are measured alone? This study also serves as a baseline assessment to compare what happens to the invaded plant communities after *A. longifolia* is removed with mechanical clearing, and after the introduction of biocontrol agents, thereby avoiding a general deficiency in this type of study as recognised by Thomas & Reid (2007).

MATERIALS AND METHODS

Study site - The study area was located in the São Jacinto Dunes Nature Reserve (hereafter SJDNR) which is located on the central-northern coast of Portugal (40º 39' N, 8º 44' W). This area has been a proclaimed nature reserve since 1979, receiving the status of Special Area of Conservation for the Natura 2000 network in 1999. When this study was initiated, SJDNR covered ca. 660 ha being enlarged subsequently. It is bordered by the Atlantic Ocean to the West and by the Ria de Aveiro estuary to the East. The climate is Mediterranean with Atlantic influence. The mean annual precipitation is 920 mm and mean monthly temperatures range from 10.2ºC in January to 20.2ºC in June. Dominant winds blow from Northwest and North, approaching from the Ocean. Historically the area supported open vegetation characterized by several plant communities composed of distinct assemblages of species (plant associations), with sporadic small trees and abundant shrubs, sub-shrubs and herbs (Neto, 1993). Such open communities still persist in non-invaded areas, including several endemic plant associations (Honrado *et al.*, 2006; Silva, 2006). *Pinus pinaster* Aiton (maritime pine) and *A. longifolia* were introduced into SJDNR early in the 20th century to curb movement of sand. *Acacia longifolia* has subsequently proliferated, occurring as a monoculture in over 150 ha and at lower levels in association with other plant species in over 200 ha (Guimarães, 2004). Besides *A. longifolia,* the reserve is also invaded to a lesser extent by *Carpobrotus edulis* (L.) N.E.Br. (ice plant) and *Cortaderia selloana* (Schultes) Asch. & Graebner (pampas grass).

The areas invaded by *A. longifolia* in SJDNR can be divided into long-invaded and recently invaded areas. Long-invaded areas (while limited to monospecific stands) have had *A. longifolia* for several decades and are largely restricted to a small area located along the Southern border of SJDNR. The recently invaded areas came about during the 1995 summer when *A. longifolia* proliferated over a large area of the reserve after a natural fire which destroyed about 200 ha of vegetation, including pine plantations and dune vegetation (Silva 1997). Before the fire, the burnt areas had low numbers of *A. longifolia* plants, mostly in the understory of maritime pines.

Experimental design - Studies were carried out to compare non-invaded, recently invaded and long-invaded areas, all located on stabilized dunes away from the sea. Non-invaded areas were free of *A. longifolia* trees, while invaded areas supported almost monospecific, arboreal stands of *A. longifolia* overlaying large quantities of leaf litter (Marchante *et al.*, 2008b) and reducing the intensity of light at soil level (Table 2.1). Open patches within the invaded areas contained remnants of the native vegetation, with several species in common with the non-invaded areas, indicating that the invaded areas had the same floral elements prior to the proliferation of *A. longifolia.*

Table 2.1 Light intensity and quantity of litter (mean ± SE) in experimental plots of long-invaded, recently invaded and non-invaded areas. Light measurements were made monthly from February to October 2006 between 9 a.m. and 12 a.m. on days without clouds. Values are means of six measurements per plot. Litter data are based on Marchante *et al.* (2008b).

	Long-invaded	Recently invaded	Non-invaded
light at soil level (µmol.m^{-2}.s^{-1})	170.2 ± 6.3	283.6 ± 9.5	1198.2 ± 28.0
litter (kg m^{-2})	2.05 ± 0.24	1.43 ± 0.14	0.55 ± 0.09

In each type of stand (non-invaded, recently invaded and long-invaded), five permanent plots, each 10 x 10 m, were demarcated. Each plot was located at least 50 m from any of the others. In each plot, two transects of 2 x 10 m was marked giving a total of 10 transects in each type of area. Transects were monitored in May/early June 2003, 2006 and 2008, when most annual and biannual species were growing (Marchante, Marchante & Freitas, 2003). The number and identity of plant species, species cover, and number of *A. longifolia* seedlings was registered in each transect.

Data analysis - Seedling abundance and cover of *A. longifolia,* species richness, total plant cover, Pielou evenness and Shannon diversity (all parameters translated as single figures) were calculated as the means of ten 20 m^2 transects and were analysed by a General Linear Model (GLM) repeated-measures MANOVA, with year of monitoring (2003, 2006 and 2008) as a within subject factor and invasion status as between-groups factor. As absence of *A. longifolia* was a prerequisite to define non-invaded areas, analyses of *A. longifolia* cover and seedling abundance did not include non-invaded areas. Data from the three sampling years were compared to detect possible changes in impacts through time. Post-hoc tests were conducted using the Fisher LSD, at $P < 0.05$. Because the interaction between factors was significant (except for Evenness) the post-hoc was performed for the interaction and the graphs were computed accordingly. Since *A. longifolia* was the potential driver of change in the rest of the plant community, it was excluded from the analysis of species richness and total plant cover. Pielou evenness and Shannon diversity calculations (Magurran, 1988) included *A. longifolia* as these indexes reflect the presence of a dominant species. STATISTICA 6.0 (StatSoft, Inc., 2001, http://www.statsoft.com) was used for the statistical analysis.

For plant traits, dominance-diversity curves, also named rank abundance curves, were computed according to Kent and Coker (1992), to describe evenness of species distribution and relative species dominance within each type of area. Curves were first computed for the full rank of species (each sampling year separately) to compare the three areas. The species were then categorized into traits (Table 2.2) and additional curves were plotted for each plant trait separately. For traits analysis the coverage of each species in the ten 20 m^2 transects were pooled to reveal the full spectrum of species in each area and then the mean of three years was computed. The species were ranked from the most to the least abundant for each curve separately.

Table 2.2 List of biological and ecological plant traits and respective attributes used for the analysis.

Trait	Attribute *	Source
Biological trait: Raunkiær life form	Therophyte, hemicryptophyte, chamaephyte, geophyte, nanophanerophytes, phanerophytes (include all phanerophytes but the nanophanerophytes)	1, 2
Biological trait: Dispersal mode #	No obvious agent; animals; wind	1, 4, 5
Ecological trait: Biogeographic distribution	Exotic & invasive, native & generalist (species that occur in several different habitats), native & dune specialist (species that are limited to dunes), native & dune/generalist (species that occur in dunes and other habitats but are limited to sandy soils)	2, 3
Ecological trait: Tolerance to soil nitrogen	Non-nitrophilous, nitrophilous, sub-nitrophilous and escionitrophilous	2, 6

1 = Field observations; 2 = Franco (1971, 1984); Franco & Afonso (1994, 1998, 2003); 3 = Marchante, Freitas & Marchante (2008); 4 = Fenner & Thompson (2005); 5 = Hodgson *et al*. (1995); 6 = Costa, Capelo & Aguiar *unpublished data.*

***** In species with more than one attribute the dominant in the studied system was considered.

In order to increase the power of the analysis if the mechanism of dispersal was not known but the species belongs to a genus/family with consistent seed dispersal across species, that species was allocated the dispersal class more typical of the genus/family.

Sorensen’s Similarity Coefficient (QS) was used to compare plant species composition in the three situations. QS detects differences between areas that have similar values of species richness, diversity or cover but are composed of different species assemblages. QS was computed with the formula $QS = 2C/(A + B)$, where A and B are the number of species in samples A and B, respectively, and C is the number of species shared by the two samples. The coefficient varies between 0 (different) and 1 (identical) (Magurran, 1988). The species present in the ten $20\ m^2$ transects were pooled, for each year separately, providing the total number of species present in the ten transects of each area.

Species turnover rates (TR) were calculated according to Hilli, Kuitunen & Suhonen (2007) to measure changes of species between 2003 and 2008, as follows: $TR = 0.5\ (L+G)$, where L is the number of species lost and G is the species gained during a defined period of time. TR is 0 (zero) when there is no change in species composition over the interval between samplings and has no maximum value when there is change.

RESULTS

Presence of *A. longifolia*

There was a significant interaction between invasion status and year of monitoring ($F_{2,10} = 4.19$, $P = 0.048$) with *A. longifolia* cover being ca. 10% higher in long-invaded areas than in recently invaded areas (Figure 2.1a). When the number of *A. longifolia* seedlings was quantified, as a measure of the reinvasion potential, there was also a significant interaction between factors ($F_{4,20} = 40.67$, $P < 0.0001$) showing a significant increase of emerged seedlings in long-invaded areas, both over time and compared to recently invaded areas. There was a different trend in recently invaded areas with seedlings decreasing from 2006 to 2008 (Figure 2.1b).

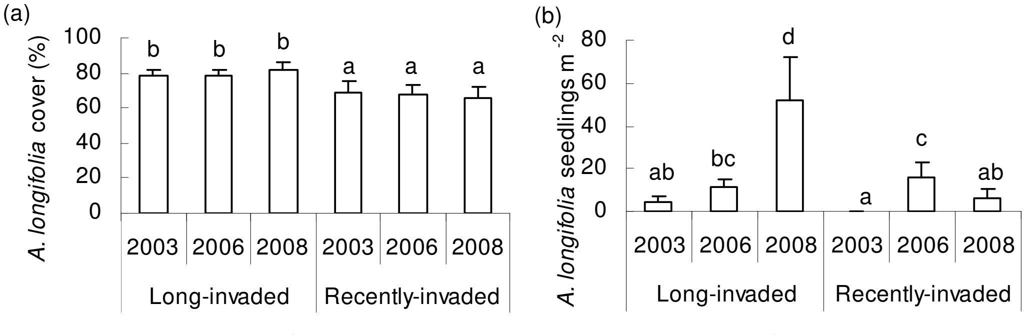

Figure 2.1 Cover of *A. longifolia* mature trees (a), and number of *A. longifolia* seedlings (b) (mean +SE, n = 10) in areas long-invaded and recently invaded by *A. longifolia,* from 2003 to 2008. Bars with the same letters are not significantly different (LSD, $p < 0.05$).

Impacts in species richness, plant cover and diversity

Overall, 75 plant species were identified during the study, including 53 species in non-invaded plots, with less than half as many (25) in long-invaded plots and 35 species in recently invaded plots (Appendix 2.1). Not surprisingly, both of the invaded areas were dominated by a single species, *A. longifolia,* while other species that were present in the invaded areas showed only very sparse cover. From 2003 to 2008, species abundances remained consistent for the most part, the exception being a marked decline in the abundance of several species in recently invaded areas (Appendix 2.2). Overall, species were more evenly distributed in non-invaded areas than in invaded areas, although two species, *Cistus salvifolius* L. and *Cytisus grandiflorus* (Brot.) DC., were more abundant in some non-invaded plots and three other species, *Carex arenaria* L., *Artemisia campestre* L. subsp. *maritima* Arcangeli and *Helichrysum italicum* (Roth) G. Don fil. subsp. *picardi* (Boiss. & Reuter) Franco were also well represented on other non-invaded plots.

Interaction between invasion status and year of monitoring was statistically significant for both species richness ($F_{4,20}$ = 5.448, P = 0.004) and plant cover ($F_{4,20}$ = 15.95, P < 0.0001). The average number of plant species per plot under *A. longifolia* canopies was less than half the number of species in non-invaded areas and it was generally similar in both invaded areas (Figure 2.2a). Plant cover, other than *A. longifolia* (Figure 2.2b), was 3 to 6-fold lower in recently- and long-invaded areas, respectively, than in non-invaded areas. Plant cover increased between 2003 and 2006 in non-invaded areas and showed some decline between 2006 and 2008 in recently invaded areas with no significant variation otherwise.

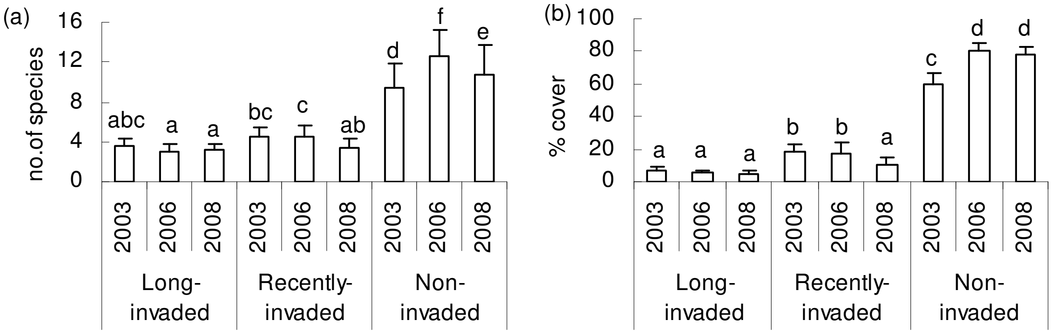

Figure 2.2 Species richness (a) and plant cover (b) (mean +SE, n = 10), in 20 m^2 plots, in areas long-invaded, recently invaded and non-invaded by *A. longifolia,* from 2003 to 2008. Values do not include *A. longifolia.* Bars with the same letters are not significantly different (LSD, $p < 0.05$).

There was a significant interaction between invasion status and year of monitoring for plant diversity (Shannon $F_{4,20}$ = 4.71, P = 0.008) but not for evenness (Pielou $F_{4,20}$ = 0.41, P = 0.798). Both factors independently had a significant effect on evenness (Invasion status $F_{2,10}$ = 183.55, P < 0.0001; year of monitoring $F_{2,10} = 18.68$, $P < 0.0001$). Both diversity measures were, at least, twice as high in non-invaded areas than in invaded areas; and higher in recently invaded areas than in long-invaded ones (Table 2.3). From 2003 to 2008, Shannon Diversity decreased in both invaded areas, while evenness decreased in recently invaded and non-invaded areas.

Table 2.3 Diversity measures (means (SE), n = 10), in areas long-invaded, recently invaded and non-invaded by *A. longifolia,* from 2003 to 2008. Values in the same column with the same letter are not significantly different (LSD, $P < 0.05$)

areas	sampling year	Pielou Evenness	Shannon Diversity
Long-invaded	**2003**	0.23(0.04)ab	0.36(0.08)b
	2006	0.21(0.03)a	0.29(0.07)ab
	2008	0.16(0.03)a	0.23(0.05)a
Recently-invaded	**2003**	0.35(0.08)c	0.64(0.16)c
	2006	0.29(0.08)bc	0.54(0.14)c
	2008	0.23(0.07)ab	0.38(0.14)b
Non-invaded	**2003**	0.74(0.06)e	1.43(0.30)d
	2006	0.66(0.05)d	1.62(0.26)e
	2008	0.63(0.08)d	1.41(0.32)d

Impacts on biological and ecological plant traits

Non-invaded areas had almost exclusively native species while several exotic species were present in invaded areas (Figure 2.3). More species that are characteristic of dunes and dune/generalists were present, and particularly abundant, in non-invaded areas, while these species comprised only about a third of all species in invaded areas. The number of native generalist species was higher in recently invaded areas, with two species co-dominating and many sparse species co-occurring.

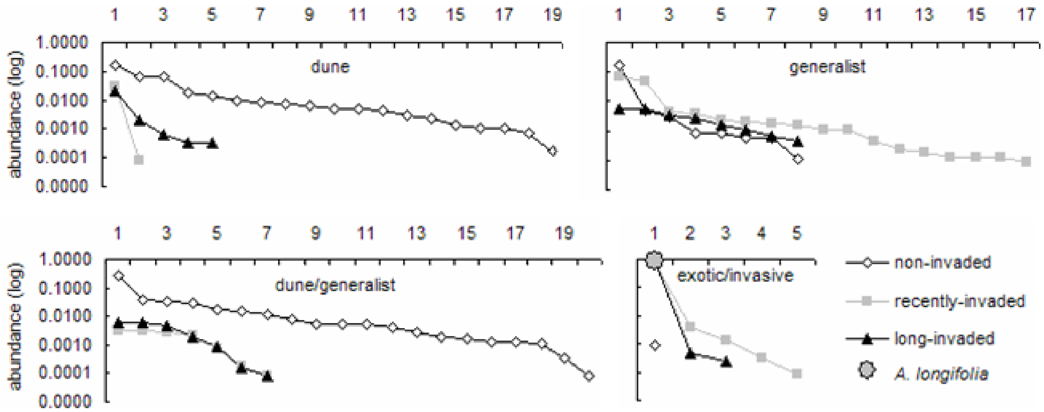

Figure 2.3 Dominance-diversity curves for each attribute of species biogeographic distribution in communities of areas non-invaded, recently invaded and long-invaded by *A. longifolia*. The sequence in each trait attribute (in abscissa) lists species from most to least abundant and consequently each number in the different sequences does not necessarily correspond to the same species. Abundance of each species plotted is an average of observations between 2003 and 2008.

Regarding life forms (Figure 2.4), nanophanerophytes were the most abundant in non-invaded areas, despite there being only five species, followed by chamaephytes (with more species evenly distributed) and geophytes (with one species particularly abundant). Invaded-areas were dominated by the (micro) phanerophyte *A. longifolia,* and the few species, very sparse, that were found under *A. longifolia* were mainly therophytes. Three life forms, namely chamaephytes, geophytes and hemicryptophytes, that were well-represented in native dunes were scarce, or even absent, in invaded areas. Phanerophytes, other than *A. longifolia,* that were present in invaded areas were in very low abundance.

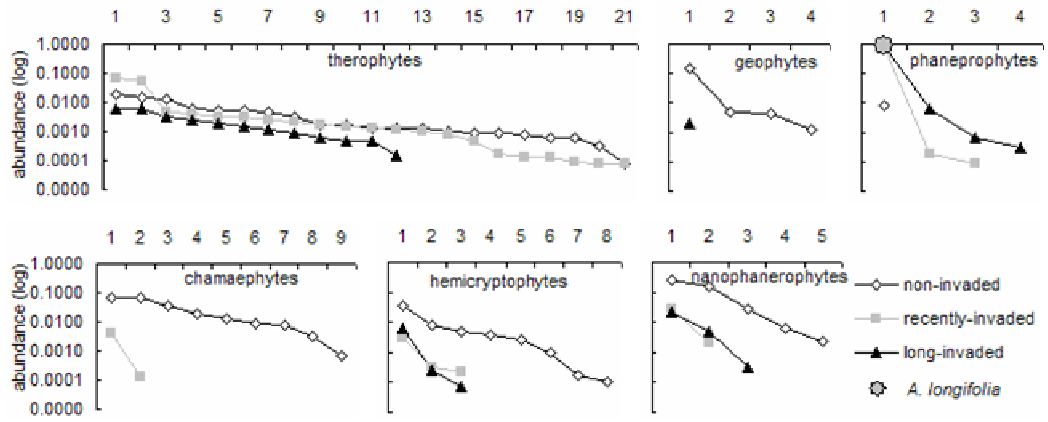

Figure 2. 4 Dominance-diversity curves for each attribute of Raunkiær life forms in communities of areas non-invaded, recently invaded and long-invaded by *A. longifolia* (see Figure 2.3 caption for details).

There was a high number of wind-dispersed species in recently invaded and non-invaded areas (Figure 2.5), with them being more evenly distributed in the latter areas. These species were apparently more sensitive to invasion age as about half were not found in long-invaded areas. Many animal dispersed species and species with no obvious agent of dispersal were excluded by *A. longifolia,* with invaded areas having less than half the numbers found in non-invaded areas.

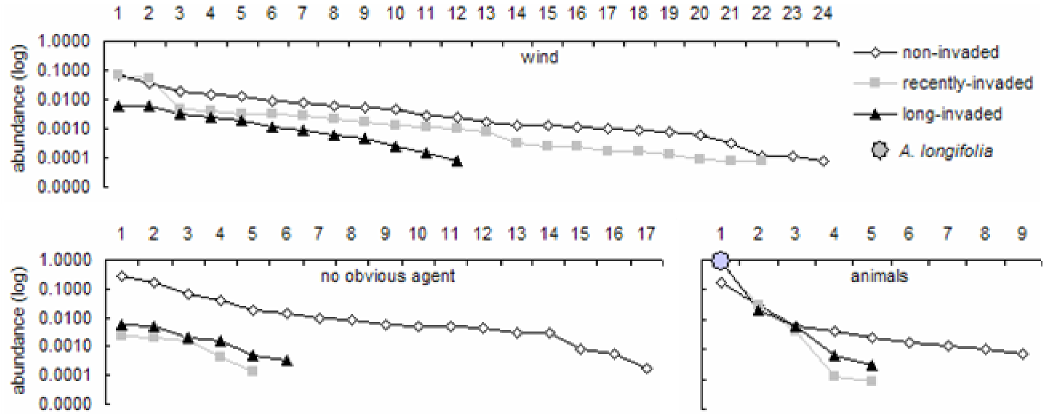

Figure 2.5 Dominance-diversity curves for each attribute of dispersal mode in communities of areas non-invaded, recently invaded and long-invaded by *A. longifolia* (see Figure 2.3 caption for details).

There were more species that are dependent on nitrogen in recently invaded areas than on native dunes, with escionitrophilous being more numerous and abundant in both invaded areas (Figure 2.6). Non-nitrophilous species were most numerous and abundant in non-invaded dunes.

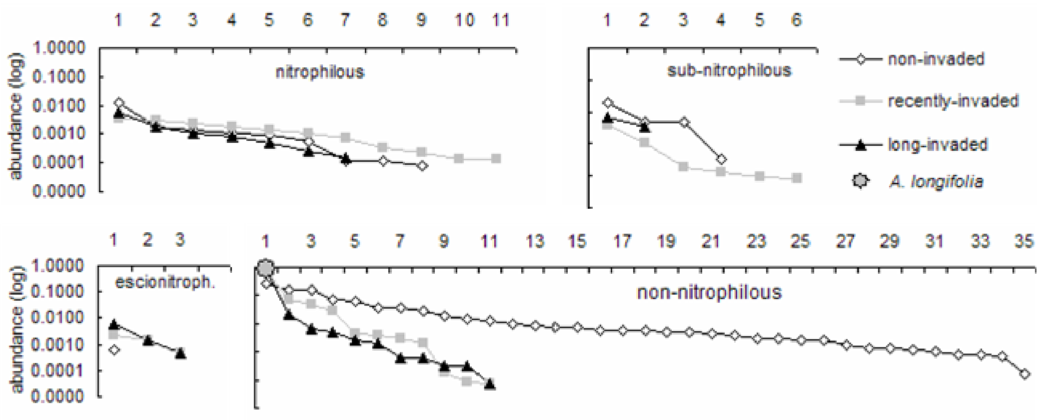

Figure 2.6 Dominance-diversity curves for each attribute of nitrophily adaptation, in communities of areas non-invaded, recently invaded and long-invaded by *A. longifolia* (see Figure 2.3 caption for details).

Similarity amongst areas

There was little similarity in species composition of invaded and non-invaded areas, decreasing with invasion duration. About 20% ($QS_{2003} = 0.18$; $QS_{2006} = 0.23$; $QS_{2008} = 0.19$) of the species were common to long- and non-invaded areas while ca. 30% species ($QS_{2003} = 0.35$; $QS_{2006} = 0.31$) were shared between recently- and non-invaded areas, with the exception of 2008 ($QS_{2008} = 0.14$). More than 50% of the species were common to both of the invaded areas ($QS_{2003} = 0.51$; $QS_{2006} = 0.56$; $QS_{2008} = 0.56$).

Species turnover rates

Turnover rates (TR) were higher than zero in all areas showing that a shift of species has occurred over time, being significantly higher in some of the areas ($F_{2,27} = 3.51$, $P = 0.044$). TR was higher in non-invaded areas (TR = 2.98 ± 0.66) and recently invaded areas (TR = 2.86 ± 0.39), which were similar to each other (P = 0.86), and higher than in long-invaded areas (TR Long-invaded = 1.46 ± 0.16; P = 0.025 and P = 0.037, when compared to non-invaded areas and recently invaded areas, respectively).

DISCUSSION

Previous short-term studies of the impacts of moderate invasions of *A. longifolia* in Portuguese dunes showed seasonal reductions in species richness which were particularly striking in spring when ephemeral life forms were most abundant, and a consistent decrease in native plant cover and diversity (Marchante, Marchante & Freitas, 2003). The present long-term study corroborates some of the earlier findings, but in areas more-densely invaded by *A. longifolia.* More importantly, the current study showed that impacts both intensify and change patterns as the time of invasion extended. Furthermore, it showed that some changes take years to become evident and that assessment of impacts including only parameters expressed by single-figures can give an incomplete picture of the changes that are occurring.

How do impacts of invasion on plant communities intensify with time?

Plant communities in areas recently invaded by *A. longifolia* were already substantially different from those in non-invaded areas showing that vegetation responds more rapidly to invasion than soil whose chemical and microbial composition was particularly altered in long-invaded stands but much less so in recently invaded areas (Marchante *et al.*, 2008b, a). Changes in plant communities were even more pronounced under long-invaded stands where the denser canopy prevents further the light penetration (Table 2.1) and increasingly reduces bare soil for other species to grow in. The thicker layer of litter accumulated on the soil surface (Facelli & Pickett, 1991; Marchante *et al.*, 2008b) and the depleted abundance of viable native seeds in long-invaded soils (Marchante, Freitas & Hoffmann, 2010a) also contributed to intensification of these changes. The cumulative degradation of plant communities with time was corroborated by the fact that there were fewer shared species between non-invaded and long-invaded areas (ca. 20%), compared with ca. 30% shared between recently invaded and non-invaded areas.

Several vegetation traits also changed with invasion time. For example, generalist species were more abundant in recently invaded areas than in long-invaded areas. The fire in the SJDNR in 1995 created gaps which suited generalist species, both native (*e.g. Crepis capillaris* (L.) Wallr., *Sonchus* spp., *Cardamine hirsute* L.) and exotic (*Gamochaeta pensylvanica* (Willd.) Cabrera and *Conyza* spp.), and the invader itself. These species are frequently associated with habitat disturbance and are uncommon or even absent in native dune communities (Alves *et al*. 1998; Franco, 1984). They are thus irrelevant (sometimes even with negative consequences) for the conservation of the system. Therophytes and wind dispersed species were less abundant in long-invaded areas. This pattern seems to be associated with: (i) the dense canopy creating a barrier to inflows of seed; and (ii) attrition of seed viability in the deep litter layers over long periods of isolation, affecting especially species that depend solely on seeds to reproduce, such as therophytes.

This demonstration that the impacts escalate and change with duration of invasion is important in that it contributes to a better understanding the invasion process (Strayer *et al.*, 2006) and, additionally, it emphasises that there are very real consequences of delaying the start of management operations against invasive plants.

What do median/long-term studies reveal about impacts of invasive plants?

Sampling repeatedly over several years enables to recognise which impacts are transient and which are permanent. In this case, germination of *A. longifolia* seeds within the thickets and replacement of species, changed over time with the changes being distinct in the different areas; short-term studies would have lost these trends. Despite the rapid proliferation of *A. longifolia* (from 0% to over 60% cover in less than ten years after the fire), during the five years of this study densities of thickets were apparently stable. Recruitment of *A. longifolia* into the populations was counter-balanced by the collapse of some mature trees (possible associated with ageing as it was more frequent in long-invaded areas) that created gaps which were quickly colonized by invasive and generalist species. Germination of *A. longifolia* seeds is typically stimulated by fire (Pieterse & Cairns, 1986) but it is also coupled with disturbance (Marchante, Freitas & Hoffmann, 2010b) as happens in many other species (Grubb, 1988; Bullock, 2000). This study showed that there is a greater threat of reinvasion in long-invaded areas, even though *A. longifolia* germination was lower in the gaps created by falling trees than in adjacent cleared areas (see chapter 5).

In long-invaded areas, parameters such as species richness, cover, evenness and diversity did not change much during the five years of the study. In recently invaded areas, these parameters decreased, with their values approaching those of long-invaded areas by the end of the study. This pattern indicates that impacts tend to stabilize over time, as corroborated by TR being lower in areas that were long-invaded, crossing a threshold at some stage as the invasion progresses.

Do single-measure parameters conceal information about impacts of invasive species?

Comparisons of species richness indicated that ca. 30% and 40% of the species in long and recently -invaded areas, respectively, persisted through the invasion. Sorensen index showed that only ca. 20% (in long-invaded) and ca. 30% (in recently invaded areas) of the species were shared with non-invaded areas, increasing the number of species that had been excluded and demonstrating that novel species had entered the invaded system. In this case at least, the single measure for species richness had not revealed the full extent of the impact of the invasion.

Although average species richness per sample plot was similar in long- and recently invaded areas, the total number of species was higher in recently invaded areas. This discrepancy can be attributed to the 1995 fire when propagules of different generalists arrived in the burnt area and emerged heterogeneously through the area. Species rank abundances revealed that the higher number of species in recently invaded stands comprised numerous species with particularly low abundances which are more likely to become locally extinct, thereby reducing species richness in these areas in a short-term, as was already evident in 2008.

Plant trait analyses showed other patterns in the impact of invasion not noticeable with single measures. The few species growing under *A. longifolia* were mostly distinct from those on non-invaded dunes. Chamaephytes, geophytes and hemicryptophytes were almost absent in the understory of invaded stands, as well as in the seed bank (chapter 4; Marchante, Freitas & Hoffmann, 2010a), reflecting the substantial threat posed by *A. longifolia* to many species that belong to these life forms and which are characteristic of dunes (*e.g. Malcolmia littorea* (L.) R. Br., *Medicago marina* L., *Crucianella maritima* L., *Herniaria ciliolata* Melderis subsp. *robusta* Chaudhri and *Iberis procumbens* Lange subsp. *procumbens*, the last two endemic) and are highly relevant for conservation of dune communities (Secretariat of the Convention on Biological Diversity, 2001). The presence of the above-mentioned species in non-invaded areas shows that, like other invasive species (Jäger, Kowarik & Tye, 2009), *A. longifolia* is unlikely to cause extinction at a landscape level. There is however cause for concern because some endemic species have been eliminated within invaded areas.

Species adapted to soils with high levels of nitrogen and/or disturbance, including exotics (e.g., *G. pensylvanica*, *Oxalis pes-caprae* L., *Conyza* spp.) and natives (*e.g. Sonchus asper* (L.) Hill, *Fumaria muralis* Koch subsp. *muralis*, *Lactuca virosa* L.) were more frequent and/or abundant in invaded areas than non-nitrofilous species that are characteristic of dunes and thrive in non-invaded communities (e.g., *Artemisia campestre* L. subsp. *maritima*, *C. maritima*, *M. littorea*). These findings confirm that invasive species not only alter the environment through their physical presence, decreasing light and space, but induce indirect impacts through altered soil chemistry and microbial changes (Ehrenfeld, 2003; Marchante *et al.*, 2008a, b). The increase in soil nitrogen and other nutrients in SJDNR (Marchante *et al.*, 2008b) may thus be compounding problems for native dune species (Smith & Huston, 1989), and at the same time facilitating *A. longifolia* and other exotics which in turn may exclude additional dune species (Warren, Topping & James, 2009; E. Marchante, *unpublished data*).

Further consequences of the impacts promoted by *A. longifolia* invasion

Pristine coastal sand dunes in Portugal are characterized by communities that extend from the ocean to the interior ordered into a spatial succession of plant associations (each with its own species assemblage) with remarkable floristic and phytocoenotic diversity (Honrado *et al.*, 2006; Silva, 2006). These species are naturally adapted to nutrient poor sands and plenty of light at soil level (Smith & Huston, 1989). Before invasion by *A. longifolia* communities with small growth habits and a few tree species sporadically present were frequent in SJDNR (Neto, 1993). Most of these protected native communities, particularly those from interior dunes, are being increasingly replaced by arboreal stands dominated by *A. longifolia,* with increasing environmental degradation (Bartz, Heink & Kowarik, 2010) and changes of ecosystem functions (Marquard *et al.*, 2009) as litter accumulates and changes soil carbon and nutrient levels (Marchante *et al.*, 2008b) and shade predominates at soil level (Table 2.1). Even so, some of the species surviving under *A. longifolia* are characteristic of native communities indicating that the invaded areas were once similar to non-invaded areas and still have some potential to be restored (see chapter 5). As with invasion by other *Acacia* species (Smith, 2010), a drastic transformation of community structure is occurring rendering an unrecognizable landscape, notably in its physiognomy and vertical stratification with loss of several strata. Because many native species do not cope with the abovementioned abiotic changes (Smith & Huston, 1989), biotic transformations are also evident, namely replacement of species and alteration of the plant traits as registered (at least partially) in other invaded systems (Holmes & Cowling, 1997; Jäger, Kowarik & Tye, 2009). As observed by Holmes (1990), animal dispersed species and species without obvious agents of dispersal (some probably also animal dispersed (Fenner & Thompson, 2005)), are commonly excluded by invading *Acacia* species, indicating that the animal seed dispersers themselves are also being excluded. This supports findings of Carvalheiro *et al.* (2010) who showed that alterations to plant communities have even more severe knock-on effects for higher trophic levels (*e.g.* herbivores, parasitoids and dispersers interlinked in ecological webs). These types of changes are almost-certainly occurring in the invaded dunes of Portugal but have not yet been investigated. Besides these trophic effects, changes in plant communities due to invasion by *A. longifolia* have repercussions for ecosystem services such as supporting (*e.g.* alteration of succession patterns and soil and nutrient cycling (Marchante *et al.*, 2008b)), provisioning (*e.g.* threats to native species, alteration of genetic resources), regulating (*e.g.*

changing pollination services and erosion and fire regimes) or even cultural services (*e.g.* effects on ecotourism, changes in perception of landscape) (Millennium Ecosystem Assessment, 2005; Vilá *et al.*, 2010). Although not quantified yet, these repercussions clearly translate into high market-related costs which need to be assessed.

This five-year study showed that invasion by *A. longifolia* in coastal dunes brings about a diversity of biotic changes in structure (decrease of species richness, cover and diversity) and function (alteration of dispersal dynamics, spectra of Raunkiær life forms and nitrogen adaptations, out-competed dune species) of the ecosystem. Some of the changes occur in short periods being lost in "snap shot" studies. Additionally, this study showed that impacts at plant communities aggravate, reinvasion potential increases and similarity to non-invaded communities' decreases as the invasion extends, apparently tending to stabilization after several decades. Trait attributes/species represented under *A. longifolia* stands change along time but that perception can be lost with analyses including only single-figures parameters.

Parallel experiments including removal of the invader and the litter beneath it will help to evaluate the recovery potential of these invaded communities (see chapter 5). In the future, economic impacts need to be assessed to show stakeholders and policy-makers the value of what is being lost.

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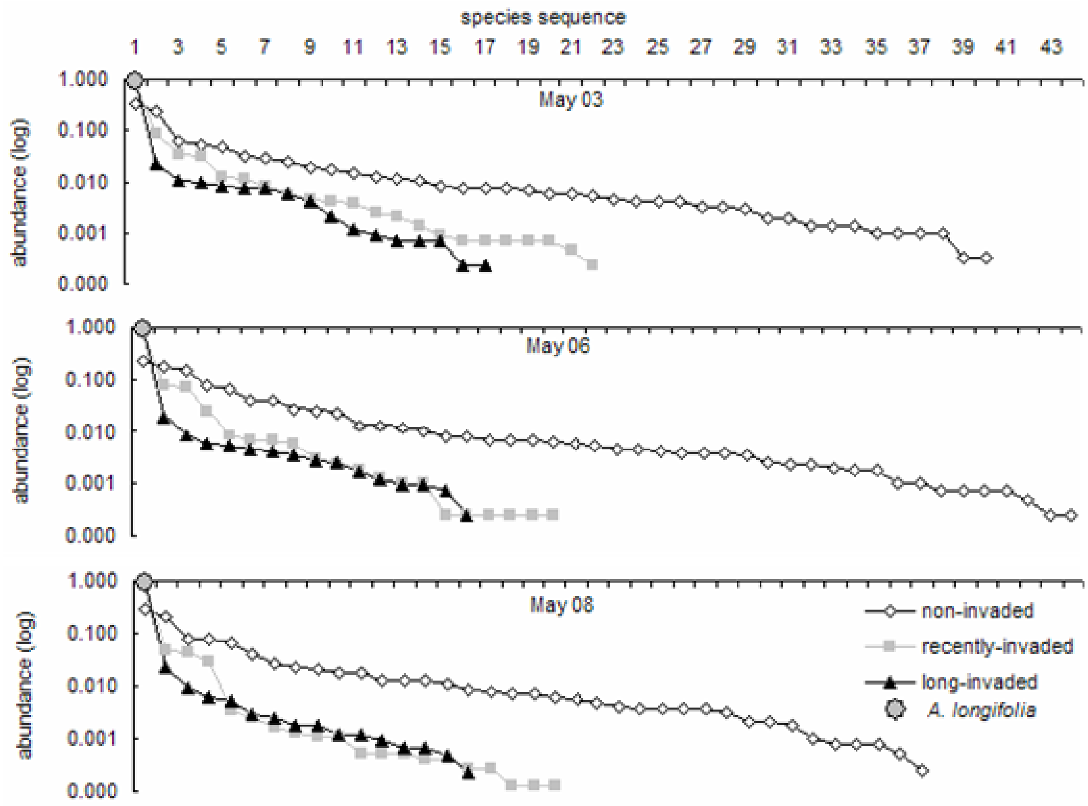
Appendix 2.1

Species identified in areas non-invaded, recently invaded and long-invaded by *A. longifolia*. Values are averages of relative abundances from 2003 to 2008.

species	family	non-invaded	recently-invaded	long-invaded
Acacia longifolia (Andrews) Willd.	*Fabaceae*	--	0.8084	0.9280
Carpobrotus edulis (L.) N. E. Br.	*Aizoaceae*	--	0.0084	--
Eryngium maritimum L.	*Apiaceae*	0.0001	--	--
Pseudorlaya minuscula (Font Quer) Laínz	*Apiaceae*	0.0018	--	--
Aetheorhiza bulbosa (L.) Cass. subsp. *bulbosa*	*Asteraceae*	0.0014	--	--
Andryala integrifolia L.	*Asteraceae*	0.0020	0.0027	--
Artemisia campestris L. subsp. *maritima* Arcangeli	*Asteraceae*	0.0606	--	--
Asteraceae 1 (not identified)	*Asteraceae*	0.0003	--	0.0003
Conyza sp.	*Asteraceae*	0.0007	0.0010	0.0004
Crepis capillaris (L.) Wallr.	*Asteraceae*	--	0.0001	--
Gamochaeta pensylvanica (Willd.) Cabrera	*Asteraceae*	--	0.0001	--
Helichrysum italicum (Roth) G. Don fil. subsp. *picardi* (Boiss. & Reuter) Franco	*Asteraceae*	0.0706	--	--
Hypochaeris glabra L.	*Asteraceae*	0.0043	--	0.0001
Lactuca virosa L.	*Asteraceae*	0.0027	--	0.0001
Logfia minima (Sm.) Dumort.	*Asteraceae*	0.0108	--	--
Picris echioides L.	*Asteraceae*	0.0008	0.0004	--
Senecio lividus L.	*Asteraceae*	0.0001	0.0028	0.0010
Senecio vulgaris L.	*Asteraceae*	0.0021	0.0026	0.0018
Senecio gallicus Vill.	*Asteraceae*	0.0176	--	--
Sonchus asper (L.) Hill	*Asteraceae*	--	0.0013	--
Sonchus oleraceus L.	*Asteraceae*	0.0004	0.0049	0.0009
Sonchus tenerrimus L.	*Asteraceae*	--	0.0001	--
Sonchus sp.	*Asteraceae*	--	0.0008	--
Urospermum picroides (L.) F. W. Schmidt.	*Asteraceae*	--	0.0048	--
Cardamine hirsuta L.	*Brassicaceae*	--	0.0030	0.0023
Hirschfeldia incana (L.) Lagrèze-Fossat	*Brassicaceae*	--	--	--
Iberis procumbens Lange subsp. *procumbens*	*Brassicaceae*	0.0088	--	--
Malcolmia littorea (L.) R. Br.	*Brassicaceae*	0.0029	--	--
Lonicera periclymenum L.	*Caprifoliaceae*	--	0.0001	--
Cerastium diffusum Pers. subsp. *diffusum*	*Caryophyllaceae*	0.0073	0.0001	--
Herniaria ciliolata Melderis subsp. *robusta* Chaudhri	*Caryophyllaceae*	0.0086	--	--
Polycarpon tetraphyllum (L.) L.	*Caryophyllaceae*	0.0091	0.0009	--
Cistus salvifolius L.	*Cistaceae*	0.2779	0.0025	0.0055
Tuberaria guttata (L.) Fourr.	*Cistaceae*	0.0028	--	--
Sedum sediforme (Jacq.) Pau	*Crassulaceae*	0.0347	--	--
Juniperus phoenicea L	*Cupressaceae*	--	--	0.0002
Carex arenaria L.	*Cyperaceae*	0.1855	--	0.0015
Cyperus capitatus Vandelli	*Cyperaceae*	0.0043	--	--
Cyperus eragrostis Lam.	*Cyperaceae*	0.0005	--	--
Corema album (L.) D. Don	*Empetraceae*	0.0018	--	0.0006
Cytisus grandiflorus (Brot.) DC.	*Fabaceae*	0.1278	0.0003	--
Lupinus angustifolius L.	*Fabaceae*	0.0042	--	--
Medicago littoralis Loisel.	*Fabaceae*	0.0017	--	--
Medicago marina L.	*Fabaceae*	0.0005	--	--
Ornithopus pinnatus (Miller) Druce	*Fabaceae*	0.0064	--	--
Stauracanthus genistoides (Brot.) Samp. subsp. *genistoides*	*Fabaceae*	0.0259	--	--
Trifolium arvense L. var. *arvense*	*Fabaceae*	0.0006	--	--
Ulex europaeus L. *latebracteatus* (Mariz) Rothm.	*Fabaceae*	0.0056	0.0277	0.0208
Erodium cicutarium (L.) LHér. subsp. *bipinnatum* Tourlet	*Geraniaceae*	0.0015	--	--
Geranium purpureum Vill.	*Geraniaceae*	--	0.0030	0.0003
Juncus acutus L.	*Juncaceae*	0.0029	--	--
Pancratium maritimum L.	*Liliaceae*	0.0043	--	--
Myrica faya Aiton	*Myricaceae*	--	--	0.0006
Fumaria muralis Koch subsp. *muralis*	*Papaveraceae*	--	0.0001	0.0067
Pinus pinaster Aiton	*Pinaceae*	0.0065	0.0001	0.0070
Plantago sp.	*Plantaginaceae*	0.0002	--	--
Agrostis stolonifera auct. lusit., non L. *pseudopungens* (Lange) Kerguélen	*Poaceae*	0.0007	--	--
Aira praecox L.	*Poaceae*	0.0006	0.0041	--
Ammophila arenaria (L.) Link subsp. *arundinacea* H. Lindb. fil.	*Poaceae*	0.0087	--	--
Briza maxima L.	*Poaceae*	--	0.0685	0.0043
Bromus diandrus Roth	*Poaceae*	0.0002	--	--
Bromus rigidus Roth	*Poaceae*	--	0.0008	0.0035
Cortaderia selloana (Schult. & Schult.F) Asch. & Graebn	*Poaceae*	--	0.0003	0.0002
Corynephorus canescens (L.) Beauv.	*Poaceae*	0.0403	--	--
Dactylis glomerata L. subsp. *hispanica* (Roth) Nyman	*Poaceae*	--	--	0.0071
Lagurus ovatus L.	*Poaceae*	0.0035	0.0001	--
Poaceae 1 (not identified)	*Poaceae*	0.0014	0.0003	0.0002
Poaceae 2 (not identified)	*Poaceae*	0.0031	0.0002	--
Vulpia bromoides (L.) S.F. Gray	*Poaceae*	--	0.0488	0.0009
Vulpia membranacea (L.) Dumort.	*Poaceae*	0.0010	--	--
Crucianella maritima L.	*Rubiaceae*	0.0134	--	--
Galium minutulum Jordan	*Rubiaceae*	--	--	0.0057
Antirrhinum majus L. subsp. *cirrhigerum* (Ficalho) Franco	*Scrophulariaceae*	0.0179	--	--
Solanum nigrum L. subsp. *nigrum*	*Solanaceae*	--	0.0001	--
Centranthus calcitrapae (L.) Dufresne subsp. *calcitrapae*	*Valerianaceae*	0.0004	0.0003	--
Magnoliopsida (seedlings) (not identified)	--	--	0.0002	--
total species richness		**53**	**35**	**25**

Appendix 2.2



Dominance-diversity curves showing the diversity in areas non-invaded, recently invaded and long-invaded by *A. longifolia* in 2003, 2006 and 2008. Abundances (logarithmic scale), on the y-axis, are plotted against species number in the sequence from most to least abundant, on the x-axis. Species in the sequence are the sum of all species present at each type of area.

Chapter 3

Seed ecology of an invasive alien species, *Acacia longifolia* (*Fabaceae*), in Portuguese dune ecosystems

ABSTRACT

Premise of the study Worldwide, invasive plants threaten biodiversity, by disrupting habitats and ecosystem processes, and cause major economic losses. Invasiveness in plants is frequently associated with prolific production of seeds which accumulate in the soil. Knowledge of the extent and persistence of invasive seed banks helps explain invasion processes and enables management planning. A study of *Acacia longifolia,* an invasive species in Portuguese dune ecosystems, provides an informative example.

Methods Seed rain and dispersal (seed traps), the persistence of seeds in the soil (burial) and the extent of seed banks were measured and analysed.

Key results Seed rain is concentrated under the canopy with ca. 12 000 seeds.m^{-2} falling annually. The number of seeds in the soil declined with time, with only 30% surviving after 75 mo. Losses were lowest at greater depths. Seeds germinability was low (<12%) but viability was high (>85%) for surviving seeds. The seed bank under the canopy was approximately 1500 and 500 seeds.m^{-2} in long- and recently invaded stands, respectively. Some seeds were found up to 7 m from the edge of stands, indicating that outside agencies facilitate dispersal.

Conclusions *Acacia longifolia* produces large numbers of seeds some of which are lost through germination, decay and granivory. The remainder form vast and persistent seed banks which serve as a source of replenishment and make it difficult to control the invader once it is established. Control costs escalate as the duration of an invasion increases, highlighting the urgency of initiating and persevering with control efforts.

Key words: invasive species managment; seed bank; seed dispersal; seed germinability; seed rain; seed viability; Sydney golden wattle

INTRODUCTION

Invasive plant species constitute a major threat to biodiversity worldwide (Richardson *et al.*, 2008; Gaertner *et al.*, 2009), disrupting the integrity and function of ecosystems (Blossey, 1999) with dire consequences for rare and endangered species. Their impacts on human health (Genton *et al.*, 2005) and economies, through management practices and losses in productivity (Lodge *et al.*, 2009), are numerous and severe and usually escalate and intensify with time (Lodge *et al.*, 2009). Very often, invasiveness in plants is associated with prolific production of seeds which accumulate in the soil and form extensive seed banks (Milton and Hall, 1981; Pieterse and Cairns, 1988; Richardson and Kluge, 2008). Successful control of alien species ultimately requires a reduction in the extent of these seed banks (Pieterse and Cairns, 1988; Richardson and Kluge, 2008) and the effectiveness of control programmes can be significantly compromised through massive seed germination in cleared areas (Campbell, 2000). Knowledge of the extent and persistence of seed banks is needed in order to understand the invasion process of each invasive species and to implement rational management programmes for their control.

To be successful, seeds have to be in the correct physiological state to germinate at a time and place that will allow the new plant to survive to maturity (Murdoch and Ellis, 2000). Seeds of some species germinate immediately after ripening, as long as water is available, while others become dormant or quiescent and remain viable for extended periods until conditions are suitable for germination (Fenner and Thompson, 2005), allowing discontinuous germination in some species (Murdoch and Ellis, 2000). There is evidence that seed longevity may vary under different environmental conditions, especially soil type and climate (Hill and Kloet, 2005) and that duration of time in the soil can extend, diminish or have no effect on the viability of seeds (Araújo and Cardoso, 2007).

Germinability of seeds may vary among species, among populations and even among individuals as a consequence of genetic, phenotypic and environmental conditions under which the seeds mature (Gutterman, 2000). These conditions may include the position of the seed on the parent plant, the age of the plant, the size of the seeds, the abiotic conditions (e.g., temperature, light, moisture, soil nutrients) where the plant develops and the position where the seeds fall (under or outside the canopy) (Gutterman, 2000). Germination, *i.e.* emergence from the seed bank, is a response to environmental stimuli (Fenner and Thompson, 2005). Particularly in hard-coated seeds, the absence of germination is generally due to physical dormancy which in many species is broken by diurnal temperature fluctuations or temperature extremes induced by fire (Baskin and Baskin, 1998).

As in other legumes, most *Acacia* species have hard-coated seeds (Cavanagh, 1980) with physical dormancy which facilitates the formation of persistent seed banks. Not surprisingly therefore, several *Acacia* species from Australia (Holmes and Cowling, 1997; Dennill *et al.*, 1999; Kutiel *et al.*, 2004a), and some from Africa (Kriticos *et al.*, 2003; NLWRA, 2008), have become notorious invasive plants in different regions of the world. One of these, *Acacia longifolia* (Andrews) Willd. (‘Sydney golden wattle’, also named ‘long-leaved wattle’), has proliferated in the dune systems of the coast of Portugal and is now considered a priority problem there (Ministério do Ambiente, 1999; Marchante *et al.*, 2003; Marchante, 2008). *Acacia longifolia* is a polycarpic species, annually producing seeds throughout its life (Fenner and Thompson, 2005). The seeds are elliptic, 4–6 x 2-2.5 mm in size, shiny (Maslin, 2001), weight 20-30 mg (Murray *et al.*, 1978); they have a small elaiosome that attracts ants, which bury and disperse the seeds over modest distances, creating a short " seed shadow" (Willson and Traveset, 2000). Despite this zoochorous adaptation in seeds of several *Acacia* species, the vast majority of seeds accumulate beneath, or very close to, the canopies of the parent plants (Milton and Hall, 1981; Walters and Milton, 2003). Seed banks of *A. longifolia* have been described as prolific and persistent in South Africa (Milton and Hall, 1981; Pieterse and Cairns, 1988). Environmental stimulation, namely fire, is generally required to break dormancy in *Acacia* species (Pieterse and Cairns, 1986) although the activities of microorganism (Trumble, 1937) and insects (Holmes and Rebelo, 1988) may also play a role.

Coastal dune ecosystems are considered a vulnerable ecosystem by International Union for Conservation of Nature (IUCN) and should have the highest priority for conservation action, namely when significant biodiversity values are at risk (Shine *et al.*, 2000). In many regions of the world, e.g., Australia (Mason *et al.*, 2007), California (Beckstead and Parker, 2003), Denmark and other NW European countries (Kollmann *et al.*, 2007), Korea (Kim, 2005), Israel (Kutiel *et al.*, 2004b), Portugal (Alves *et al.*, 1998; Rodríguez-Echeverría *et al.*, 2007; Marchante *et al.*, 2008), Spain (Sobrino *et al.*, 2002), studies with different perspectives show that invasive plant species modify, or have the potential to affect (Kollmann *et al.*, 2007), coastal ecosystems in many different ways, including degradation of biodiversity. Moreover, the invaders frequently belong to life forms previously underrepresented in several habitats of dune ecosystems, thereby causing major transformations of the vegetation structure (Mack, 2003; Marchante *et al.*, 2003) and even loss of habitat diversity.

Although *A. longifolia* is an aggressive invader in several ecosystems, including dunes, around the globe (Henderson, 1995; Weber, 2003; Elorza *et al.*, 2004; Marchante *et al.*, 2008), studies of its seed bank and the potential threat it constitutes for dune ecosystems after removal of the invader are scarce. Particularly in Portugal, there have been no studies on the seed banks of invasive species and on their role in the invasion process in dune (or other) ecosystems. In order to redress this deficiency and to compare the situation in Portugal with other parts of the world, and thereby assist in the management of *A. longifolia,* we measured: 1) the seed rain and dispersal from the plants; 2) the survival and viability of seeds after entering the seed bank; and 3) the extent of the resultant accumulated soil seed bank. Understanding the longevity of seeds allows estimations to be made of the short- and long-term probability of reinvasion in areas that have been cleared of invasive species, while knowledge of patterns of seed rain can provide valuable information about dispersal capacity and determine the extent to which non-invaded areas are at risk (Cottrell, 2004).

MATERIALS AND METHODS

Study site - Experiments were conducted in São Jacinto Dunes Nature Reserve, a coastal sand dune ecosystem of approx. 660 ha, at the central-northern coast of Portugal (40º 39' N, 8º 44' W). The climate is Mediterranean with Atlantic influence, with a mean annual precipitation of 920 mm and mean monthly temperatures ranging from 10.2ºC in January to 20.2ºC in June; mean maximum temperatures are 24.2ºC in August and mean minimum temperatures are 6.0ºC in January (Weather Station no.102, Aveiro University 40º 38' N, 8º 40' W, period 1981-2002). Dominant winds blow from Northwest (NW) and North (N), approaching from the Ocean. The soils are arenosols according to FAO classification (Rogado *et al.*, 1993). Historically the area supported open vegetation characterized by sporadic small trees and more abundant shrubs, sub-shrubs and herbs. Early in the 20th century *A. longifolia* was deliberately introduced to curb sand erosion and over time it proliferated and spread from areas where it was first introduced with the increases in density and extent occurring in pulses triggered by fire events (Silva and Lopez, 1997). When the experiments were carried out, *A. longifolia* constituted a near-monoculture in over 150 ha and occurred at lower densities in association with other species in over 200 ha (Guimarães, 2004).

The existing stands can be divided into long-invaded areas, where the plant has been established for several decades, and recently invaded areas, where the plant has proliferated within the past 15 yr. The long-invaded areas (limited to stands with monocultures of *A. longifolia*) are restricted to a small portion of the reserve located along the south border where the sands are stable (Figure 3.1). In long-invaded areas dense thickets persist for more than 20 yr. *Acacia longifolia* proliferated in the recently invaded areas after a natural fire in the 1995 summer which affected about 200 ha of the reserve, including pine plantations and dune vegetation (Silva, 1997). Before the fire, burnt areas had sporadic *A. longifolia* plants in low densities, frequently in the understory of *Pinus pinaster* Aiton. Since the fire, *A. longifolia* has invaded large areas that extend from the primary dune to the stabilized dunes (Marchante, 2001) (Figure 3.1). When the experiments were undertaken, *A. longifolia* was the dominant species in both the long-invaded and recently invaded areas selected, forming continuous closed stands (frequently with a cover about 70%) with native plant species occurring infrequently in the understory (Marchante *et al.*, 2004).

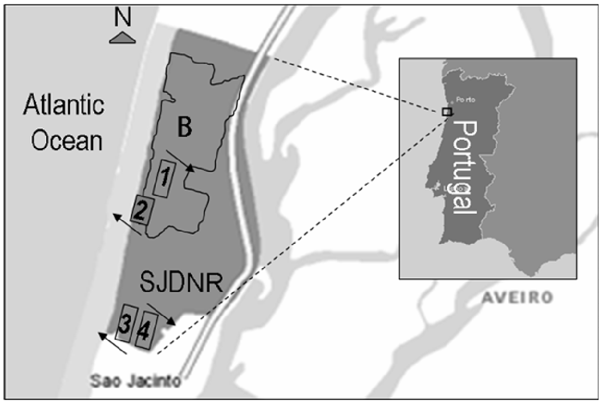


Figure 3.1 Location of the study area, São Jacinto Dunes Nature Reserve, in central-northern Portugal, showing areas where experiments of dispersal and soil seed bank took place. Numbers indicate places where transects were demarcated, in recently invaded areas (1, leeward transects and 2, windward transects) and long-invaded areas (3, windward transects and 4, leeward transects). Arrows indicate the orientation of transects in each area. B, area burnt in 2005, and largely invaded after that (recently invaded areas).

Experiment 1. Seed production and dispersal - Other studies, and personal observations, showed that *Acacia* seeds/ seedlings are frequently detected in close proximity to the stands of parent plants so the experimental design was set up accordingly. To determine the possible influence of wind on dispersal, two sets of trap transects were set out so that each was aligned with direction of the prevailing winds and at a right angle to the edge of a recently invaded stand of *A. longifolia.* Six of the transects were placed on the leeward side of the thicket, which happened to be on the stabilized dune, and six were placed on the windward side of the thicket, which happened to be on the primary dune (Figure 3.1 – no. 1 and 2, respectively). In the primary dune *A. longifolia* plants were small (about 1.5 m tall) and prostrate due to exposure to strong winds and salt spray from the sea. The plants were much taller (more than 5 m) on the stabilized dunes. Another set of six transects was established in the stabilized dunes at right angles to the edges of a long-invaded thicket to compare seed fall there with that in the recently invaded area (Figure 3.1 – no. 1 and 4, respectively). The long-invaded and recently invaded stands of *A. longifolia* were about 2.5 km apart.

Seed traps were set out at the end of May 2004, before seeds started to fall, to quantify seed rain and evaluate dispersal mediated by wind and/or gravity. As the source was a continuous stand, a regular distribution of traps was used (Bullock *et al.*, 2006). The traps were placed at intervals along permanent transects, 12 lying outside the stand (points designated “+”) and 5 inside the stands (designated “–“) with the point under the tip of the extreme pod-bearing branch set as point zero (Figure 3.2a). From -2 m to +2 m, seed traps were spaced 0.5 m apart; from +2 m to +10 m and from -2 m to -3 m the traps were a meter apart. Seed traps (Figure 3.2b) were made from the top portion of laterally bisected 5 l plastic bottles that were inverted to make a funnel with a 0.0154 m^2 opening.

A fibreglass bag was tied around the neck of the funnel to form a closed container in which the falling seeds were trapped. Each funnel was positioned in the field by being attached, with flexible wires, to a 10 cm high PVC pipe which was partially sunk into the sand, after Cottrell (2004).

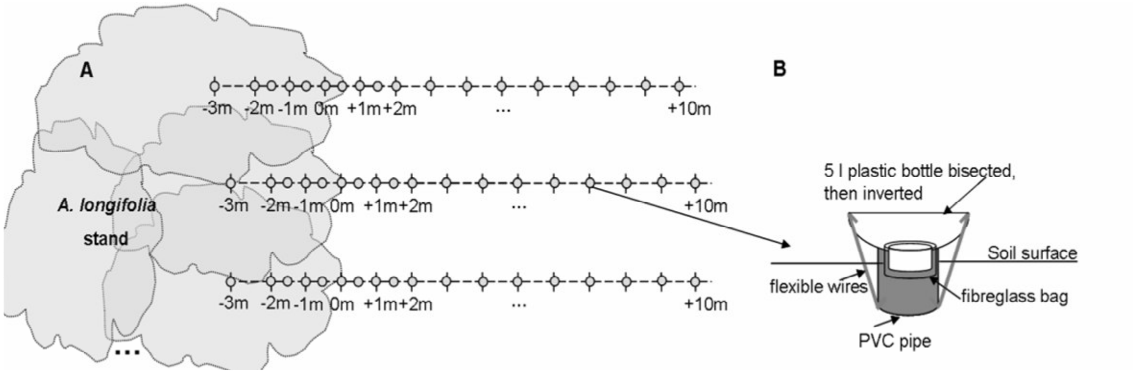


Figure 3.2 Experimental set up of experiment 1. (A) Schematic representation of transects established to measure seed rain and dispersal, with 10 m lying outside the stand (0 to +10 m) and 3 m inside the stand (0 to -3 m); the point at the extremity of pod-bearing branches was set as point zero; 1 m intervals were used, but from – 2 m to + 2 m additional traps were placed at 0.5 m (o). (B) The seed trap, designed to catch *A. longifolia* seeds. Traps were partially sunk into the sand to avoid drift into the trap of seeds lying on the surface; fibreglass allowed free drainage.

Seeds were collected from the traps at fortnightly intervals after seed rain commenced in June 2004 and continued until seed fall stopped (end of August 2004). The seeds were counted, and then checked for germinability and viability (see methods below).

Experiment 2. Survival of seeds in soil seed bank – Using a protocol adapted from Holmes and Moll (1990), ripe seeds were collected during July 2002 from dehiscent pods of *A. longifolia* trees for burial at different depths within cylinders made of fibre glass. The cylinders were filled with soil from the study site which had been freshly sieved (2 X 2 mm) to remove any extraneous seeds of *A. longifolia*. While being filled, each cylinder had one hundred seeds of *A. longifolia* sown at one of three depths, 1-2 cm, 4-5 cm or 8-9 cm from the surface. Once filled with soil, a plastic net lid (2 x 3 mm) was attached to seal the open end of the cylinder. To protect the cylinders from disturbance by burrowing animals, each was enclosed in a plastic net sleeve (10 cm deep x 7.5 cm diameter) which was sealed at both ends. The mesh of the net (2 x 3 mm) was large enough to allow seedlings to emerge. Each cylinder was labelled with a plastic tag indicating the position of the seeds within. The cylinders were buried with their tops leveled with the soil surface.

The cylinders were placed in position, after the peak of seed fall, in open spaces among the native dune vegetation, where there were no *A. longifolia* trees, in order to avoid additional deposition of seeds in the top of the cylinders. A completely randomised block design was used to position groups of cylinders in five different locations each marked with a wooden stake in the NE-corner. A map of the positions of the cylinders was drawn to facilitate recollection. In all, 96 cylinders were buried (6 collecting dates x 5 replicates x 3 depths + 6 controls with no seeds, to confirm that seeds were not entering the cylinders after placement).

Cylinders were collected after 4, 10, 18, 31, 55 and 75 mo. Any seedlings observed emerging from the bags at collecting times were recorded and removed. On each collection date, 15 bags with seeds, as well as one of the control bags, were lifted and transferred to the laboratory where the contents were sorted with a sieve to obtain the remaining seeds which were counted. Surviving seeds were tested for germinability and viability (see methods below).

In order to analyze the data, seeds in each sample were considered as 1) emergent (germinated in the field); 2) germinable (germinated in the laboratory without stimuli); 3) dormant (germinated after stimulation); 4) non-viable (rotted or did not germinate for other reasons); or 5) missing. Seeds defined as “recovered” included viable and non-viable with seeds defined as “viable” including germinable and dormant.

Experiment 3. Dimension of soil seed bank -- Areas adjacent to the dispersal experiment (1) were selected and monitored. The long-invaded areas were located on stabilized dunes while the recently invaded areas included both primary dune (along the windward edge of the thicket) and stabilized dune (along the leeward edge) (Figure 3.1).

Transects were laid at a right angle to the edge of thickets on both the windward and leeward sides. The transects (n = 4 for each situation) were 10 m long (Figure 3.3). The point under the tip of the most extreme pod-bearing branch was set as point zero so that 7 m of transect extended away from the stand (points designated “+”) and 3 m extended into the stand (designated “-“).

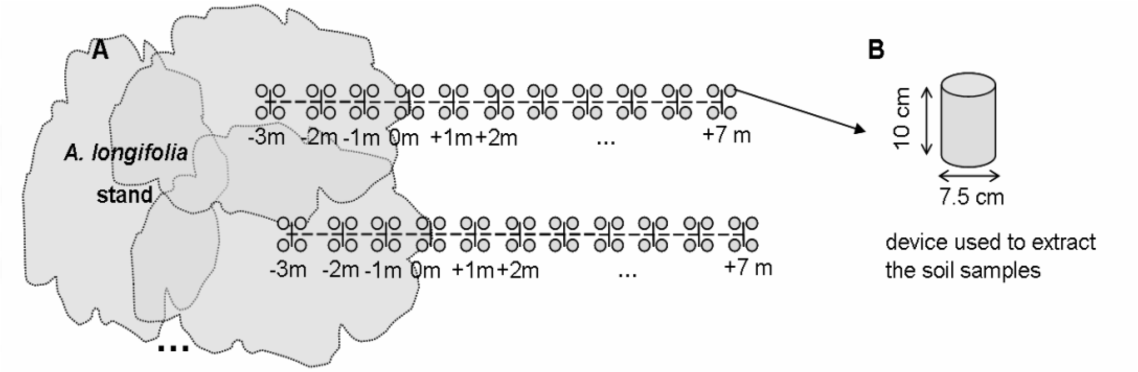


Figure 3.3 Experimental set up of experiment 3. (A) Schematic representation of transects established to measure the seed bank, with 7 m lying outside the stand (0 to +7 m) and 3 m inside the stand (0 to -3 m); the point at the extremity of pod-bearing branches was set as point zero. (B) Coring device used to extract soil samples; four samples were collected in each point.

Before seed rain commenced (7 to 14 April 2004) soil samples were collected along the transects to quantify the accumulated seed bank of *A. longifolia.* At meter intervals, four soil cores (7.5 cm diameter X 10 cm deep; area 44 cm^2) were collected and sieved (2 mm mesh) to extract and count *A. longifolia* seeds. All of the seeds were retained and screened for germinability and viability (see methods below).

Germinability and viability determination - To test germinability seeds were placed in 90 mm sterilized, glass Petri dishes containing filter paper moistened with benomyl solution (0.022% active ingredient benlat), to avoid fungal development, and retained in a controlled environment at 25 (± 2)ºC. Germinated seeds (those with at least 2 mm radicle) and rotten seeds were counted every two days and removed. After 30 days the remaining seeds were considered to be dormant and were cut at the micropylar end and maintained until all seeds had germinated or rotted to determine total viability. Seeds that germinated after cutting were considered dormant and were added to the seeds that germinated before cutting to derive the total number of viable seeds.

Statistical analyses – The counts of seeds resulting from the seed rain (experiment 1), and accumulated in the seed bank (experiment 3), were converted into number of $seeds.m^{-2}$ before analysis. Seed rain (experiment 1) was first compared between recently invaded transects running in opposite directions (one factor), with distance to the stand margin (another factor), using a General Linear Model (GLM), with a between-subject design Factorial ANOVA. The samples from the leeward transects were compared with another Factorial ANOVA, considering stand age and distance to the stand margin as factors. The quantity of seeds accumulated in the soil seed bank (experiment 3) was compared with a Three-Way Factorial ANOVA with age of invasion, wind direction, and distance to the stand margin as factors. In experiment 1 and 3 (separately) all seeds originating from samples collected at each distance were pooled together in each group (long- and recently invaded; leeward and windward) and germinability and viability calculated and then compared with One-Way ANOVA.

In the burial experiment (2), seeds recovered were compared with a GLM, with a between-subject design Factorial ANOVA (depth and duration of burial as factors). The values of germinable and viable seeds were then analysed as percentages of the recovered seeds using a Generalized Linear Model (GLZ), with the number of recovered seeds as an offset term in order to accommodate sample size.

When the interactions between factors were significant, means of the interactions were plotted; when the interactions were not significant the means were plotted for each factor separately.

Data were transformed when necessary to meet the ANOVA assumptions: seeds in the seed bank, (experiment 3) were log transformed ($\log(x+2)$); and seeds dispersed (experiment 1) were square root transformed ($\sqrt{x+0.5}$). Mean differences were separated with Tukey test at 5% level of significance. The analyses were performed using STATISTICA 6.0 (StatSoft, Inc. 2001, www.statsoft.com).

RESULTS

Seed production and dispersal -- Collection of falling seeds showed that there was a significant interaction ($F_{7,\,80}$ = 5.06, P < 0.001) between wind direction and distance to the stand margin. After being released from the pods, the majority of the seeds fell under the trees (-3 m to 0 m), reaching average values of ca. 12 000 seeds.m^{-2} in the leeward transects and less than 2000 seeds.m^{-2} in the windward transects (Figure 3.4). A few seeds fell beyond the margin of the stand, being dispersed up to 1 m in both leeward and windward transects. There was little difference in the quantity and pattern of seed rain along the leeward edge of long- and recently invaded thickets ($F_{1,\,100}$ = 3.13, P = 0.080) (Figure 3.5). Only a small proportion of the seeds fell beyond the immediate edge of the canopy, and no seeds were found more than 2 m from the canopy.

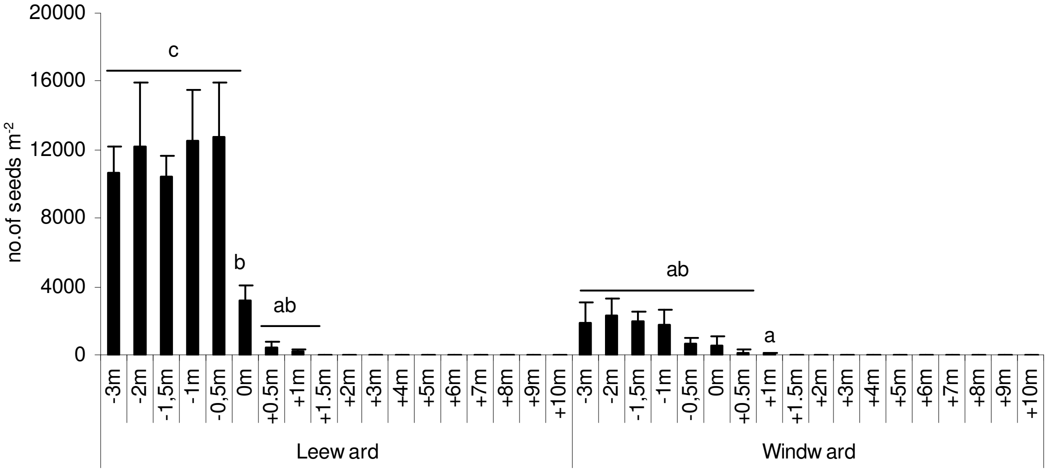


Figure 3.4 Comparison of *A. longifolia* seed rain and subsequent wind dispersal occurring along the leeward and windward edges of recently invaded thickets. Collections were made along transects extending from - 3 m to + 10 m, with 0 m being the extremity of pod-bearing branches. Bars (means +SE, n = 6) with the same letters are not significantly different (Tukey test P < 0.05).

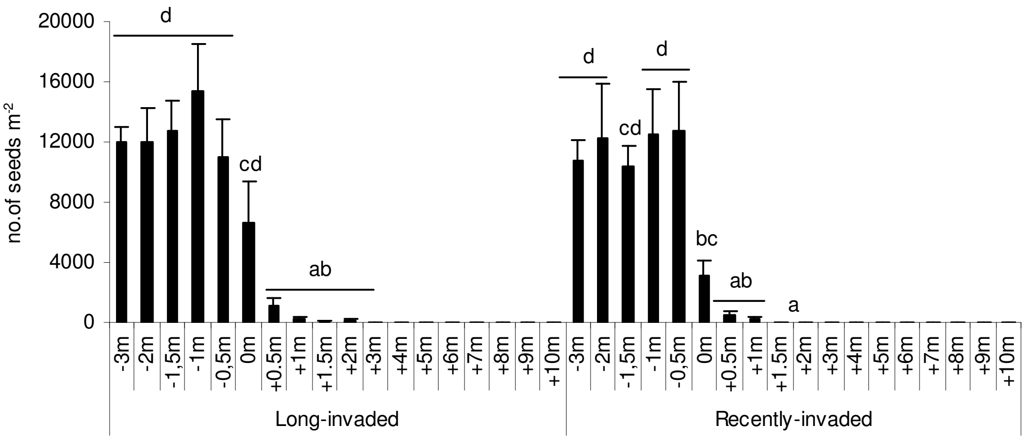


Figure 3.5 Comparison of *A. longifolia* seed rain and subsequent wind dispersal occurring along the leeward edge of long-invaded and recently invaded thickets. Bars (means +SE, n = 6) with the same letters are not significantly different (Tukey test $P < 0.05$).

Seed germinability immediately after ripening was about 30-40% (Figure 3.6a, c), indicating that approximately 60-70% of the *A. longifolia* seeds showed innate dormancy at the time of dispersal. Most of the seeds needed stimulation to induce germination. Seed viability was high (about 90%) regardless of the situation in which they fell (Figure 3.6b, d). Neither the age of the invasion nor the position of the trees relative to the wind influenced germinability (age, $F_{1, 10} = 0.159$, $P = 0.698$; position, $F_{1, 10} = 0.182$, $P = 0.679$) and viability of the seeds produced (age, $F_{1, 10} = 1.305$, $P = 0.280$; position, $F_{1, 10} = 0.065$, $P = 0.804$) (Figure 3.6).

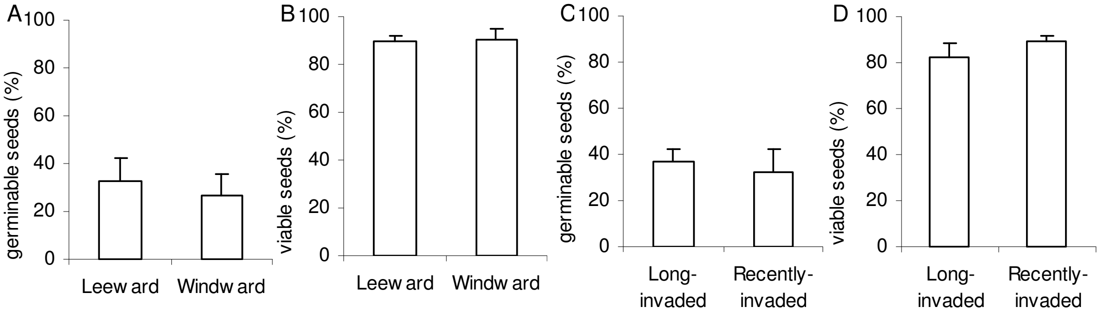


Figure 3.6 Measurements of *A. longifolia* seeds that germinated without any stimulus (germinability) (a, c) and after being stimulated (viability) (b, d), after the seed rain from plants along the windward and leeward margins of the thickets (A, B) and along the leeward margin of long-invaded and recently invaded thickets (C, D). Values are expressed as a percentage of the number of seeds collected in the traps. Bars are means +SE. There was no significant difference between any of the treatments.

Survival of seeds in soil seed bank - There was no significant interaction between duration and depth of burial ($F_{10, 77} = 1.57$, $P = 0.132$); both factors independently showed a significant effect (duration of burial, $F_{5, 77} = 34.31$, $P < 0.001$; depth, $F_{2, 77} = 12.53$, $P < 0.001$) on the amount of seeds recovered after burial. The percentage of *A. longifolia* seeds recovered decreased with duration of burial (Figure 3.7a). The decline in the number of seeds recovered was significant after 18 mo and again after 55 mo of burial. After 75 mo of burial less than 30% of seeds were recovered. Seeds buried at 8-9 cm showed significantly lower losses than at more superficial depths (Figure 3.7b). In the field, seedlings were seldom seen and emergent seeds were only observed when collections were made at 4 mo, in very low numbers (at the most, 4 seedlings were observed per cylinder) and only from the 1-2 cm and 4-5 cm depths. No seeds were found in the control samples.

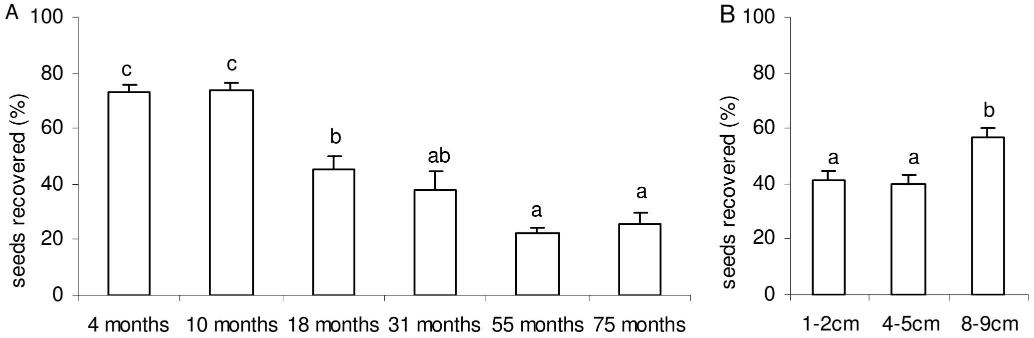


Figure 3.7 Percentage of seeds of *A. longifolia* recovered after burial (a) for different durations and (b) at different depths. Bars (means +SE, n = 5) with the same letters are not significantly different (Tukey test $P < 0.05$).

The germinability of the buried *A. longifolia* seeds was consistently low, rarely reaching 12% of the recovered seeds (Figure 3.8). Even so, time (GLZ: $_{\text{Wald stat. } 4}$ = 154.03, $P < 0.001$) (Figure 3.8a) and depth (GLZ: $_{\text{Wald stat. } 2}$ = 14.76, $P = 0.006$) (Figure 3.8b) of burial was statistically significant. After 10 mo of burial there was an increase in the seeds that germinated without any stimuli. The slightly higher germination apparent at 75 mo was distorted due to an unavoidably-small sample size, not being considerably different from the others.

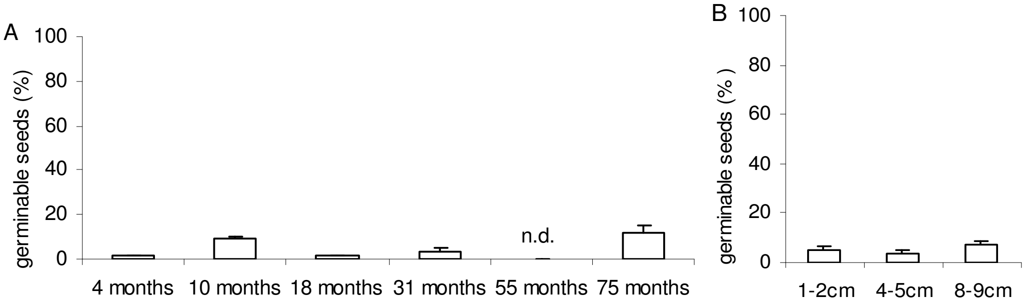


Figure 3.8 Percentage of *A. longifolia* seeds that germinated without any stimulus (germinability) following (a) different burial durations and (b) burial at three depths. Bars are means +SE. n.d. = no data.

Although there was some statistical significance in the variation of viability at different depths (GLZ: $_{\text{Wald stat. } 2}$ = 12.05, $P = 0.002$) and times (GLZ: $_{\text{Wald stat. } 5}$ = 156.87, $P < 0.001$) of burial, most of the *A. longifolia* seeds recovered were viable but dormant (*i.e.* requiring a stimulus to germinate) with viability persistently > 85% for all situations (Figure 3.9).

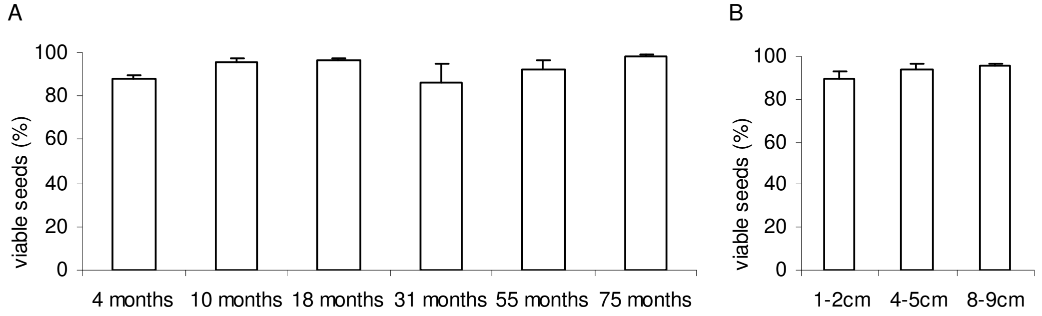


Figure 3.9 Effect of (a) depth of burial, and (b) duration of burial on the percentage of seeds of *A. longifolia* that germinated after being stimulated (viability). Bars are means +SE.

Dimension of soil seed bank - In the counts of seeds in the soil, significant interactions were found between age of invasion and distance to the stand margin ($F_{10,\,264}$ = 3.06, P = 0.001), and between wind direction and distance to the stand margin ($F_{10,\,264}$ = 2.35, P = 0.011). There was a close to significant interaction between age of invasion and wind direction ($F_{1,\,264}$ = 3.83, P = 0.051). The accumulation of *A. longifolia* seeds in the soil was almost exclusively under the trees (-3 m to -1 m), with significantly higher numbers in the long-invaded areas ($F_{1,\,264}$ = 16.41, P < 0.001) (Figure 3.10). There were more seeds in the soil under trees on the windward side of the long-invaded thickets than under the trees on the windward side of the recently invaded thickets.

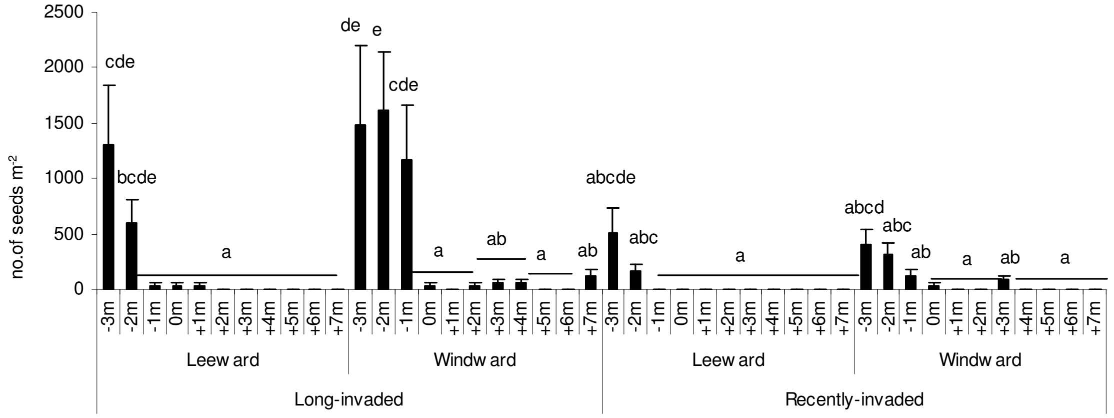


Figure 3.10 *Acacia longifolia* accumulated seed banks considering stands of different ages (long-invaded & recently invaded), different wind directions (windward & leeward) and distance to the stands margin. Bars (means +SE, n = 8) with the same letters are not significantly different (Tukey test P < 0.05).

The extent of the seed bank under the trees (-3 m to -1 m) varied between ca. 1500 seeds.m^{-2} in long-invaded areas, to less than 500 seeds.m^{-2} in recently invaded. Outside the stands the seed bank was substantially lower, rarely reaching 30 seeds.m^{-2}. Seeds were recovered at a greater distance on the windward sides of the thickets (7 m and 3 m from the edges of the long-invaded areas and recently invaded areas, respectively) than on the leeward sides where seeds were found at 1 m from the edge of only the long-invaded areas.

Germinability was significantly higher (about 65%), and dormancy rate consequently lower, in seeds from recently invaded soils than in seeds from long-invaded soils ($F_{1, 5}$ = 299.56, $P < 0.001$) (Figure 3.11a) rates of which were negligible. Total viability (Figure 3.11b) of *A. longifolia* seeds was significantly higher in seeds from recently invaded soils ($F_{1, 5}$ = 7.38, $P = 0.042$), where it was close to 100%, compared to about 70% in seeds from long-invaded areas.

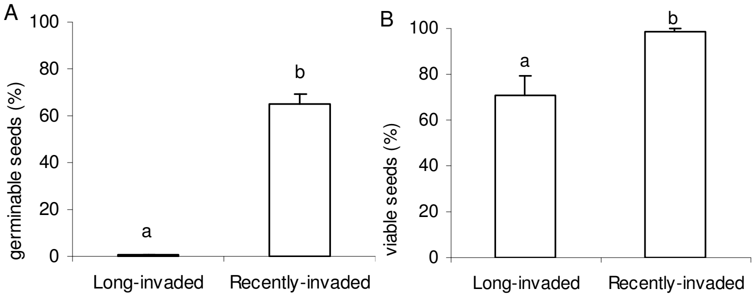


Figure 3.11 Measurement of *A. longifolia* seeds accumulated in the seed bank (a) that germinated without any stimulus (germinability) and (b) after being stimulated (viability), expressed as a percentage of total number of seeds collected in soil samples. Bars with the same letters are not significantly different.

DISCUSSION

The levels of seed production that were measured revealed that *A. longifolia* is highly prolific in this exotic location and that, despite major losses of seeds (both before and after entering the seed bank), the resulting seed banks are substantial. This accumulation of seeds create problems for management programmes because they germinate *en masse* following removal of the overstory and the cleared areas rapidly become replenished thereby increasing control costs and impeding the recovery of ecosystems towards a more natural state.

Seed production and dispersal - Seeds of *A. longifolia* fell mainly under the canopy, with very few being detected beyond 1 m, regardless of transect direction, a pattern which is common for many species, including other *Acacia* (Walters and Milton, 2003), that are not typically wind dispersed (Bullock and Clarke, 2000). Gravity, and not the wind, seems to be the agent operating in the movement of the great majority of seeds, although some seeds were found in the soil up to 7 m beyond the edge of the windward side of the thickets. Seeds of *A. longifolia* are known to be dispersed by ants, which are attracted to the elaiosome (Willson and Traveset, 2000), and have been observed carrying seeds in the Portuguese dunes. Other outlying seeds could be the result of periodic strong winds blowing in the opposite direction to the dominant winds (Bullock and Clarke, 2000) or may have been moved by other animals, such as rodents (Holmes, 1990), whose activity was not incorporated into the sampling process (Cottrell, 2004), or birds. *Acacia longifolia* seedlings have been reported under nests of collared dove (*Streptopelia decaocto*) in the vicinities of SJDNR (R. Vaz, SJDNR*, personal observation*), supporting this last hypothesis. Occasional seeds have been moved considerable distances because isolated individuals of *A. longifolia* occur in locations considerably distant (*i.e.* 100s of meters) from the main thickets (H. Marchante*, personal observation*).

Trees on the leeward side of the thickets (wind NW-SE), were located in a landward location, on stabilized dunes, protected from strong winds and salt spray. They were more upright and produced more seeds than windswept trees making this aspect the predominant direction of invasion. Trees in both long and recently invaded areas produced similar quantities of seeds indicating that, as in other species (Fenner and Thompson, 2005), the quantity of seeds produced is not significantly influenced by the age of the plant. Up to 16 000 (average 12 000) seeds.m^{-2} fall under the trees on the leeward side of the thicket in a single season. Studies with the same species in South Africa (Milton and Hall, 1981) measured lower numbers of seeds (approximately 3000 seeds.m^{-2}) but this discrepancy may have occurred because there are substantial annual fluctuations in levels of seed production (Shibata *et al.*, 2002), or due to inadvertent seed loss from the traps. The lower number of seed in South Africa may also be due to the type of trap used, since seed predation and/or removal of seeds by wind are probably higher in mesh traps than in funnel traps used in our study (Kollmann and Goetze, 1998).

Survival of seeds in soil seed bank – Our results show that once buried only 20-40% of *A. longifolia* seeds persisted in the seed bank after 31 mo, with little change after 75 mo. Studies with *Acacia saligna* and *Acacia cyclops* showed that, after 30 mo of burial at depths between 1 and 10 cm, 49-79% of *A. saligna* seeds persisted in the seed bank while less than 5% of *A. cyclops* did so (Holmes and Moll, 1990). *Acacia longifolia* shows a pattern more similar to that of *A. saligna*. Holmes and Moll (1990) reported that survival declined more rapidly with deeper burial while we observed the opposite in accordance with other studies (Zorner *et al.*, 1984; Lonsdale *et al.*, 1988). If shallow seeds disappear more rapidly, it is expected that seeds will persist for longer in areas with deep sandy soils because more seeds will reach greater depths. These findings should be considered when planning management of such areas.

Several factors contribute to seed loss. Germination naturally contributes to seed bank declines even though Fenner and Thompson (2005) note that the majority of seeds fail to emerge as seedlings. In this study, the few *A. longifolia* seedlings that were detected emerging in the field were from seeds at depths above 4-5 cm. Other species have been shown to emerge from greater depths (Kent *et al.*, 2001), including *A. saligna* and *A. cyclops* whose seedlings reach the soil surface from depths of 15 cm (Holmes and Moll, 1990). For both these species, seedling emergence in the field was generally much higher than for *A. longifolia* and germination continued for at least 30 mo after burial (Holmes and Moll, 1990). In the experiments reported here, some *A. longifolia* seedlings may have been overlooked, having appeared and died in the period between sample collections. Other studies with *A. longifolia* in the same dune system showed that numerous seeds germinate in the field (from unknown depths), mainly, as happens with many plant species (Grubb, 1988), after disturbance associated with the removal of the parent plants, with most dying soon after emergence (Marchante *et al.*, 2004).

As buried samples were not disturbed, and vestiges of seedlings were not detected on the soil surface, germination does not seem to be the main reason for disappearance of seeds. Unsuccessful germination at depths too deep to emerge has also been implicated as a major reason for seed losses from seed banks (Lunt, 1995). In this study, some signs of germination were apparent as vestiges of radicles or seed coats in the soil samples recovered but disintegration and fragmentation made it impossible to extrapolate the original numbers of intact seeds involved, a difficulty noted by other authors (Bekker *et al.*, 1998). Nevertheless, mortality through unsuccessful germination at excess depths does not seem to be the main cause of seed loss, as most seeds disappeared at shallower depths.

Decay through the action of microrganisms must also have played a prominent role in determining numbers of surviving seeds, as has been implicated with other *Acacia* species (Holmes, 1989) and legume species in general (Leishman *et al.*, 2000). Death due to natural ageing does not seem a probable cause of decline because the seeds were buried for only 75 mo and *Acacia* species are known to have higher longevities in the soil (Cavanagh, 1980; Daws *et al.*, 2007).

Low germinability in batches of seeds stored in the soil has been shown for *A. saligna* and *A. cyclops* (Holmes and Moll, 1990). The higher germination rate of *A. longifolia* seeds collected after 10 mo of burial could be due to the seeds being collected in spring, when temperatures were increasing and there was still sufficient rainfall to keep conditions in the soil moist and therefore favourable for breaking dormancy. Dormancy of seeds is known to break during periods of increasing ambient temperatures (Holmes and Moll, 1990), especially, at least in legumes, if there is simultaneous absorption of water through the hilum of the seeds (Degreef *et al.*, 2002). Most of the other collection times fell in winter, when temperatures were lower and the seeds had not been stimulated to break dormancy before collection.

The resulting soil seed bank – *Acacia longifolia* plants in long- and recently invaded areas produced similar amounts of seeds (average 12 000.m^{-2}, under the canopy) each season but there were substantially lower numbers of seeds in the soil seed banks, with more in long-invaded areas than in recently invaded areas (approximately 1500.m^{-2} and 500.m^{-2}, respectively). This differs from the situation in South Africa where there were more seeds in the seed bank (7646 seeds.m^{-2}) than the numbers produced each season (ca. 3000 seeds.m^{-2}) (Milton and Hall, 1981). A portion of the discrepancy that lead to the higher densities of *A. longifolia* seeds in South African soils is attributable to the value 7646 being an average of two means, 2110 seeds.m^{-2} and 13182 seeds.m^{-2}, which were collected 6 mo after and immediately after seed rain, respectively (S. Milton, University of Stellenbosch, *personal communication*). The lower of these values is still higher than the values recorded during our study and suggests a substantial and steady loss of seeds in the months following seed rain, as our samples were collected just before seed rain commenced. Indeed, as seeds accumulate with time the seed bank would be expected to numerically exceed its source of replenishment, as happened in South Africa. The unexpected deficit in the seed bank in Portugal may be due to conditions in the dune ecosystem that favour the decay of seeds or climatic conditions that induced higher levels of germination.

Events that happen before the seeds enter the seed bank (germination, dispersal, granivory) and within the soil seed bank (decay, ageing, germination, granivory) account for seed disappearing. The probability of some of these events happening within the seed bank in Portugal have been discussed above. Granivory, which has been shown to be a cause of seed loss elsewhere (Marone *et al.*, 2000), would not account for the loss of seeds in burial trials because the samples were protected with plastic net sleeves. However, granivory could explain a significant proportion of seeds that were lost, both before entering and within the seed bank. Granivores, namely rodents, have already been identified as a major consumer of seeds of *A. cyclops*, *A. saligna* (Holmes, 1990) and *A. longifolia* (Pieterse and Cairns, 1988) in South Africa even though granivory declines as dense thickets of the weed form and the canopy closes (Holmes, 1990). Fieldmice (*Apodemus sylvaticus*) were sporadically observed during trials (H. Marchante*, personal observation*) and are a potential seed predator of *A. longifolia* seeds. Other, as yet unidentified, invertebrates are known to feed on the seeds of *A. longifolia* (E. Marchante, University of Coimbra, *unpublished data*).

The more numerous soil seed bank under long-invaded stands can be explained by the greater number of seed rain events from trees that had been in the area for a longer period than the trees in the recently invaded area. Studies with *A. saligna* have similarly shown that in some areas seed density is much higher in long-invaded sites compared to recently invaded sites, though this is not always the case (Holmes, 2002).

Seeds of *A. longifolia* fell, and subsequently accumulated, predominantly under the canopy of the parent trees. A few scattered seeds were additionally found in the soil beyond the margins of the thickets even though dispersal trials failed to detect seeds in the tail of the dispersal curve, as is often the case (Portnoy, 1993). In order to account for this, sampling could have been intensified with distance (Bullock *et al.*, 2006). The outlying seeds could be the result of the action of several dispersal agents (see discussion above).

Most of the seeds gathered during the different parts of this study did not germinate without a stimulus, displaying low levels of germinability, but had consistently high levels of viability (> 85%), as previously recorded by Milton and Hall (1981). A large portion of fresh *Acacia* seeds are impermeable to water and are therefore unlikely to germinate soon after ripening (Rolston, 1978). Germinability levels of fresh seeds recorded in this study were higher (approximately 30%) than the 2% recorded for fresh seeds of *A. cyclops* and for several other *Acacia* seeds extracted from soil, namely *A. longifolia,* where germinability never exceeded 9% (Milton and Hall, 1981). The age of the parent plant had no apparent effect on germinability of fresh seeds even though germinability is known to decline with age of the parent in some plant species (Gutterman, 2000). Seeds collected from long-invaded areas had much lower germinability (< 0.05%) than seeds collected from recently invaded areas (> 50%). This indicates that over time dormant seeds accumulate in the soil so that older seed banks have a greater proportion of seeds that are unable to germinate without being stimulated, *i.e.* usually through disturbance. While the seed banks of long-invaded areas contain higher numbers of seeds than more-recently invaded areas, they constitute a smaller threat of replenishing the original stands of the weed through germination if disturbance of the soil can be avoided during removal of the overstory. However, zero disturbance is almost impossible to achieve given the nature of control operations and the fact that removal of the overstory in itself is likely to provide stimuli that will break dormancy.

Hilhorst and Karssen (2000) highlight the importance that physical and chemical environments have on the germination of seeds. There are several differences in the biotic and abiotic conditions of areas with native vegetation (where seeds were experimentally buried in experiment 2) and areas invaded by *A. longifolia* (where seeds are naturally buried in the seed bank, experiment 3). Light at soil level and soil temperature are higher in non-invaded areas, while moisture and accumulated litter are higher in long-invaded areas with recently invaded areas always having intermediate values (E. Marchante, *unpublished data*). Nutrient contents (C, N, Ca^{2+}, Na^{+}, K^{+} and Mg^{2+}) and microbial activity in the soil are generally higher in invaded than in non-invaded areas (Marchante *et al.*, 2008). After entering the seed bank, seeds remain dormant until the testa becomes weathered and water-permeable (Rolston, 1978). Different factors, such as N content or microbial activity, contribute to this process. Another cause of differential level of germinability has been noted in other *Acacia* species (Holmes and Moll, 1990) in that the hardness of the seed coat in each cohort of seeds is determined by the conditions under which the seeds mature.

CONCLUSIONS

In spite of the substantial and continual attrition in seed numbers during and following seed fall and in the soil seed bank, there is a substantial reservoir of viable seeds under and around *A. longifolia* thickets. These banks are the source of reinvasion after any clearing operations. Seeds in the soil remain viable for at least six years so the pool persists even if control operations disrupt recruitment of new seeds. Our findings show that managers have to be prepared to take immediate action after disturbances (e.g., fire or clearing operations) that cause changes in the coat properties of seeds and set off germination en masse. If nothing is done, the situation will worsen after disturbance.

The results of this study also showed that seeds continue to accumulate in the soil with time and that the longer nothing is done about an invasion the more likely that there will be extensive and prolonged recruitment of new plants after initial clearing operations, requiring more resources to deal with the problem. Neglecting the problem will also allow invaded stands to advance by approximately 1 m every 2-3 yr (*i.e.* the age at which new plants first set seeds) while some seeds will be dispersed greater distances, creating new invasion foci (Moody and Mack, 1988) representing a threat to non-invaded areas, as already confirmed (Marchante *et al.*, 2010; see chapter 4). Additional experiments are currently being conducted to identify the agents actively involved in long-range dispersal of *A. longifolia* seeds in these systems.

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Chapter 4

The potential role of seed-banks in the recovery of dune ecosystems after removal of invasive plant species

ABSTRACT

Question: How resilient is the seed bank of an invaded dune system? Is that resilience dependent on duration of invasion? How does the accumulated litter layer contribute to the soil seed bank?

Location: Coastal sand dunes invaded by *Acacia longifolia,* Portugal.

Methods: Seedling emergence was used to quantify and compare soil seed banks in long-invaded, recently invaded and non-invaded areas. Changes in seed banks were also compared to areas where *A. longifolia* and the litter layer were removed.

Results: Species richness, seedling density and diversity were higher in non-invaded and recently invaded areas, than in long-invaded areas. Although there was an apparent similarity between non-invaded and recently invaded areas, analyses of species traits revealed differences. Non-invaded areas had a wider array of traits. Exotic/invasive species dominated invaded seed banks while native species dominated non-invaded seed banks. Life forms, growth forms, longevity and dispersal mode showed differences between areas, with cleared plots of long-invaded areas being apparently the most similar to non-invaded plots. *Acacia longifolia* seeds were most abundant in long-invaded areas, particularly where the litter layer remained. Removal of *A. longifolia* plus the litter had little effect on the seed bank composition of recently invaded areas but resulted in noticeable changes in seed banks of long-invaded areas.

Conclusions: Long-invaded areas are less resilient and show a higher reinvasion potential, despite severe alteration of the seed banks of both areas. Seed bank studies can be a useful tool to guide management, but can give misleading results when invasion periods are protracted.

Key-words: *Acacia longifolia;* diversity; dune ecosystems; resilience of invaded ecosystem; plant traits; seedling emergence.

Nomenclature source: Franco (1971, 1984) and Franco & Afonso (1994, 1998, 2003) for plant species.

INTRODUCTION

Invasive species are currently considered one of the main threats to biodiversity worldwide (Rejmánek & Richardson 1996; Richardson & Pyšek 2008). Their impacts include declines in populations of native species (Chornesky & Randall 2003) and changes in ecosystems functioning

(Vitousek & Walker 1989; D'Antonio & Kark 2002; Peperkorn *et al.* 2005); they also have economic impacts (mainly through control efforts and decreasing productivity of agricultural land) (Pimentel *et al.* 2005) and there may be related health issues. In Portugal, as in other parts of the world, the problem is particularly acute in natural and semi-natural conservation areas (Coblentz 1990; Cowie & Werner 1993; Dana *et al.* 2003; Marchante *et al.* 2004; Aguiar *et al.* 2007; Foxcroft *et al.* 2007). Although control of invasive species is a priority, successful rehabilitation of natural habitats is ultimately determined by the extent to which native species are able to re-establish. Natural recovery of native flora in cleared areas depends largely on the availability of propagules, as well as abiotic conditions that favour their germination and survival (De Graaf *et al.* 1998).

The soil seed bank includes viable seeds that are present in both the soil and associated litter layer (Simpson *et al.* 1989). Assessing seed bank attributes (species richness and composition, species guilds and seed density) can help to reveal the resilience of invaded ecosystems and to determine whether natural regeneration will suffice for rehabilitation or whether other interventions are required. Changes in structure are considered to be a sensitive measure of changes in a community (Elzinga *et al.* 2001). According to Frieswyk & Zedler (2006), a resilient seed bank has seed densities typical of the original system, with many species from many guilds, and a high proportion of native species. On the other hand, a degraded seed bank has atypical seed densities, few native species or guilds, or a high proportion of exotic species.

Several authors - see Bossuyt & Honnay (2008) and references there in - are sceptical that restoration based on germination from soil seed banks can be successful and note that seed banks in invaded areas are usually dominated by low numbers of species. Nevertheless, other authors note that accumulations of dormant seeds allow native plants to persist through many years of invasion (Sternberg *et al.* 2003) and facilitate recovery in cleared areas. In Mediterranean climates the dominant species in the seed bank are annual plants that show adaptive responses to the unpredictable nature of the environment they inhabit by producing many seeds. Although the composition of aboveground communities does not necessarily reflect the abundance of species in the soil seed bank, in frequently-disturbed areas there is often a close correlation between adult plant species and species of seeds in the soil (Baptista & Shumway 1998). In densely invaded systems, seed bank studies can reveal species that have the potential to play a key role in the recovery of the ecosystem after control of the invasive species. The findings can be considered positive when they show that native species are well represented, and negative when undesirable species are predominant. Such information can be decisive when planning to embark on an expensive control programme.

Coastal dune ecosystems are considered a vulnerable ecosystem type by the International Union for Conservation of Nature and should have the highest priority for conservation action when significant biodiversity values are at risk (Shine *et al.* 2000). Due to stressful conditions (*e.g.* low soil fertility and water availability, sand movement, strong winds and salt spray from the sea) dune ecosystems have distinct phytocoenosis which are ecologically distinct (Carter 1995) and include high proportions of endemic species and habitats (Honrado *et al.* 2006). They are particularly vulnerable to disturbance through natural perturbations and human activities (Hanson & Lindh 1993; Carter 1995). The stability and natural dynamics of these ecosystems relies on their diversity of native plant species which bind the sand and curb erosion (van der Putten & Peters 1995). Along the Portuguese coast, pristine dune systems are becoming increasingly rare, with native species being replaced by several invasive exotic species (Alves *et al.* 1998). One of these species is *Acacia longifolia* (Andrews) Willd which threatens plant diversity, changes vegetation structure (Marchante *et al.* 2003; see chapter 2) and alters ecosystem functioning (Marchante *et al.* 2008b, c).

Although *A. longifolia* is an aggressive invader in several ecosystems, including dunes, around the globe (Henderson 1995; Weber 2003; Elorza 2004; Marchante *et al.* 2008b), studies quantifying its impacts on natural seed banks and on the resilience of dune systems are scarce. Additionally, there is a general lack of data on soil seed banks of coastal sand dunes (Owen *et al.* 2001). In order to determine the role of seed banks in the restoration of invaded communities, and the susceptibility of such communities to reinvasion, the seed banks of areas that had been long- and recently invaded by *A. longifolia* were assessed and compared with non-invaded areas. An assessment was also made of the effect of removing *A. longifolia* plants with the underlying litter layer on the residual seed bank. We hypothesised that: 1) in degraded natural ecosystems that have been overrun with invasive species the potential for recovery declines with age of invasion because the composition of seed banks changes as seeds are removed from the system, through granivory, failed germination or decay, without being replenished; 2) most of the seeds of the invasive species will be near the soil surface so that removal of the litter layer along with the parent plants will reduce the capacity for reinvasion of cleared areas and; 3) the composition of native species will differ in situations where leaf litter is removed compared to areas where litter is left intact because seeds of some species occur predominantly in the surface layers while others occur at depth in the soil.

More generally, the study serves as an example which should be of use to the many organisations globally that are responsible for the management of invasive plant species in natural areas. It shows the type of research that is required, and the kind of questions that should be asked, to get the information that is needed to understand the dynamics of the mix of seeds in the system and the relative effects of different types of control operations on these seeds. Doing so will enable managers to develop control methods which will maximise removal of the invader while fostering seeds of desirable species along with abiotic conditions that favour their germination.

MATERIAL AND METHODS

Study area - The study area was located in the São Jacinto Dunes Nature Reserve (SJDNR) which is situated on the central-northern coast of Portugal (40º 39' N, 8º 44' W). The reserve covers about 660 ha and is bordered by the Atlantic Ocean to the West and by the Ria de Aveiro estuary to the East (location provided at the Appendix 4.1). The climate is Mediterranean with Atlantic influence, a mean annual precipitation of 920 mm and mean monthly temperatures ranging from 10.2ºC in January to 20.2ºC in June. Dominant winds blow from Northwest and North, approaching from the Ocean. Historically the area supported open vegetation characterized by shrubs, sub-shrubs and herbs and sporadic small trees. The reserve has been extensively invaded by *A. longifolia* which threatens the high conservation value of the region (Marchante *et al.* 2004; Marchante *et al.* 2007). Besides *A. longifolia,* the reserve has also been invaded to a lesser extent by *Carpobrotus edulis* (L.) N.E.Br. (ice plant) and *Cortaderia selloana* (Schultes) Asch. & Graebner (pampas grass).

Acacia longifolia was introduced into SJDNR early in the 20th century to curb movement of sand in coastal areas, has subsequently proliferated in several areas and has invaded about two thirds of SJDNR. The stands in SJDNR can be divided into long-invaded and recently invaded areas. Long-invaded areas have had dense stands (over 80% cover) of *A. longifolia* for several decades and are largely restricted to a small area located along the South border of the SJDNR, where the sands are stabilized. The recently invaded areas came about during the 1995 summer when *A. longifolia* proliferated (with cover reaching 70%) over a large, previously-unaffected area of the reserve after a natural fire which destroyed about 200 ha, eliminating pine plantations and dune vegetation (Silva 1997) in both the primary and stabilized dunes. Before the fire, the burnt areas had low numbers of *A. longifolia* plants, mostly in the under-story of *Pinus pinaster* Aiton.

Large areas of SJDNR support almost mono-specific stands of *A. longifolia* trees which are causing a significant change in the community structure (Marchante *et al.* 2003; chapter 2) and ecosystem functioning (Marchante *et al.* 2008b; c). These changes include 1) replacement of diverse plant communities, dominated by herbs, sub-shrubs and shrubs species (Neto 1993; Honrado *et al.* 2006), with arboreal stands largely dominated by *A. longifolia* with native plant species occurring infrequently in the understory (chapter 2), and 2) the deposition of large quantities of leaf litter ranging from (mean ±SE) 2.05 ± 0.24 $Kg.m^{-2}$ in long-invaded areas to 1.43 ± 0.14 $Kg.m^{-2}$ in recently invaded areas as opposed to 0.55 ± 0.09 $Kg.m^{-2}$ in non-invaded areas (Marchante *et al.* 2008b).

Experimental design - To assess the effects of the *A. longifolia* invasion on the composition of the soil seed bank, two experiments were performed:

Effect of invasion status on the seed banks – the seed bank composition in areas recently invaded and long-invaded by *A. longifolia* was compared with non-invaded areas. Non-invaded areas were selected inside SJDNR on the basis that: a) they were in a comparable dune situation (interdune, stabilized dune) to the invaded areas; and b) there were no *A. longifolia* trees in the area. Small open areas within the invaded areas contain remnants of the native vegetation, with several species in common with the non-invaded areas, indicating that they had the same floral elements and seed banks prior to the invasion by *A. longifolia.* In each area five 10 X 10 m plots were established from which soil samples were collected (see below).

Effect of A. longifolia and litter removal on the seed banks - in both the long-invaded and recently invaded areas a complete randomized block design was used to define five blocks, each consisting of two 10 X 10 m plots. Each pair of two plots was similar before treatments application. One of two treatments was randomly assigned to one plot in each block. The treatments were: a) plots cleared of *A. longifolia* by cutting the trees with chainsaws at ground level after which the leaf litter layer was removed (designated the 'ALR' treatment); and b) untreated plots with both *A. longifolia* and litter left intact (designated the 'A' treatment) (these were the same plots that were sampled in experiment 1 above). Trees and litter were removed in October 2002 and seed bank samples were collected in December 2002.

Seedling emergency method was used to assess the composition of seed banks. There are some known limitations with the method (*e.g.* species whose seeds require specific stimuli to germinate may not be detected) but these are outweighed by the advantages, namely that it readily eliminates inviable seeds, which can confound the results, and that seedlings are usually more easy to identify than seeds. In each plot, three soil samples (15 samples per treatment; cylindrical cores 7.5 cm in diameter and 10 cm deep) were collected. A total volume of 6623 cm^3 of soil was collected from a surface area of 662 cm^2 within each treatment. The soil was then sieved (1 cm mesh) to extract stones, coarse roots and other plant material before being spread on a layer of sterilized subsoil in a tray (10 x 20 cm in area; 5 cm deep). Trays were kept in a green house with regular irrigation (twice a day) and ambient light and temperature conditions. Germinating seeds were registered fortnightly and seedlings were grown until positive identification was possible. The plants were then removed and preserved as herbarium specimens (Herbarium from Escola Superior Agrária de Coimbra). A set of control trays was installed with a layer of sterilized subsoil only and the seedlings that appeared in these were used to correct numbers in the experimental trays for dates after appearance. Seedling emergence was monitored until no seeds germinated for at least 13 months (January 2004).

Data analysis

Species richness, seedlings density and quantity of A. longifolia seedlings - were analysed separately for each experiment. For these three parameters data from the three trays of each plot were first gathered in a composite sample. Data are presented per 133 cm^2 which corresponds to the area of three soil cores. To evaluate the effect of the invasion status (experiment 1) on each parameter a General Linear Model (GLM), with a between-subject design One-Way ANOVA was performed. The effect of invasion age and clearing treatment (experiment 2) was evaluated with a GLM, with a between-subject design Factorial ANOVA. Significant differences detected were then located with the LSD post-hoc test at 5% level of significance. Data on seedlings density of both experiments had to be log transformed ($\log(x+1)$) to fulfil ANOVA assumptions (Zar 1996). Seedlings that died before positive identification were included in the measures of seedling abundance but not in the analyses of separate species. All the statistical analyses were performed using STATISTICA 6.0 (StatSoft, Inc. 2001, www.statsoft.com).

Seedlings diversity and similarity between seed banks – these parameters were analysed, and are presented, together (experiment 1 and 2 combined) in order to avoid data repetition. Shannon diversity index was calculated for each invasion status and clearing treatment and compared using a *t* test proposed by Hutcheson (1970); the index of Shannon ranges from 0 to ≈ 5, but is usually found to fall between 1.5 and 3.5 (Magurran 1988).

Similarity between seed banks regarding species composition at different invasion status and treatments were analysed with Sorensen´s Similarity Coefficient (($QS = 2C/(A+B)$, where A and B are the species numbers in samples A and B, respectively, and C is the number of species shared by the two samples), which varies between 0 (different) and 1 (identical).

Functional traits - the seedlings were additionally categorized into functional and other traits (Table 4.1). For every treatment and invasion status, the number of seedlings with each attribute was summed and divided by the total number of seedlings to produce a relative abundance of seedlings with each attribute. *Acacia longifolia* seedlings and all the other species were treated separately in the analyses. Seedlings that did not survive to a stage of development where they could be identified to the species level were allocated to attributes whenever possible; the remaining seedlings were classified as “undetermined”.

For calculation of these three parameters, species abundances of all trays of each invasion status and treatment were first summed to get a total value of abundances per species.

Table 4.1 List of plant functional traits and other categories and attributes used for the analysis of changes.

Trait	Attribute *	Source
Raunkiær life form	Therophyte, hemicryptophyte, chamaephyte, geophyte, nanophanerophyte, microphanerophyte	1, 2
Growth form	Graminoids (include grasses and sedges), herbs, shrubs, trees	1, 2
Longevity	Short-lived (annual or biennial), perennial (life cycle over more than 2 years)	1, 2
Main dispersal agent #	No obvious agent, animals, wind	1, 4, 5
Origin & habitat	Exotic & invasive, native & generalist (species that appear in several different habitats), native & dune specialist (species that are limited to dunes), native & dune/generalist (species that despite appearing in more habitats than dunes are limited to sandy habitats)	2, 3
Taxonomy	Families represented	2

1 = Field observations; 2 = Franco (1971, 1984); Franco & Afonso (1994, 1998, 2003); 3 = Marchante *et al.* (2008a); 4 = Fenner & Thompson (2005); 5 = Hodgson *et al.* (1995)

* In species with more than one attribute the dominant in the studied system was considered. In all traits the attribute “undetermined” was created to include seedlings that did not survive until identification and additionally were not able to be safely allocated to another attribute.

In order to increase the power of the analysis in investigating seed dispersal, a species that had unknown mechanism of dispersal, but belongs to a genus/family with consistent seed dispersal across species, was allocated the dispersal class of the genus/family.

RESULTS

Species richness, seedlings abundance and quantity of *A. longifolia* seedlings

Overall, 1322 seedlings representing 42 taxa germinated in the trays, including 39 identifiable species, two species that could only be placed at genus level and one species that could only be placed at family level (Appendix 4.2). Of these, 182 did not survive long enough to allow identification. The unidentifiable seedlings had morphological characters which indicated that they represented predominantly three species and a rare fourth species. Only one species (*Salix atrocinerea* Brot.) germinated in the control trays (four seedlings in all) and this figure was used to adjust counts in the sample trays. Most of the species were herbs, predominantly from *Asteraceae* (in all areas) and *Caryophyllaceae* (in recently invaded areas). Two graminoid taxa, *Poaceae* (in recently invaded area) and *Juncaceae* (in non-invaded areas), were also represented by several species. Although only five exotic species were identified, three of these were very abundant, namely *Conyza* sp., *Gamochaeta pensylvanica* and *A. longifolia* (Appendix 4.2).

Effect of invasion status on the seed banks – The density of seedlings was more than double in recently invaded and non-invaded areas than in long-invaded areas ($F_{2,12} = 6.08$, $P = 0.015$) (Figure 4.1a). Species richness was also affected by invasion status ($F_{2,12} = 3.86$, $P = 0.050$) with long-invaded areas having fewer species than recently invaded areas (Figure 4.1b). The seed banks of long-invaded areas had ca. 4-fold more *A. longifolia* seedlings than the seed banks of both recently- and non-invaded areas ($F_{2,12} = 5.76$, $P = 0.018$) (Figure 4.1c).

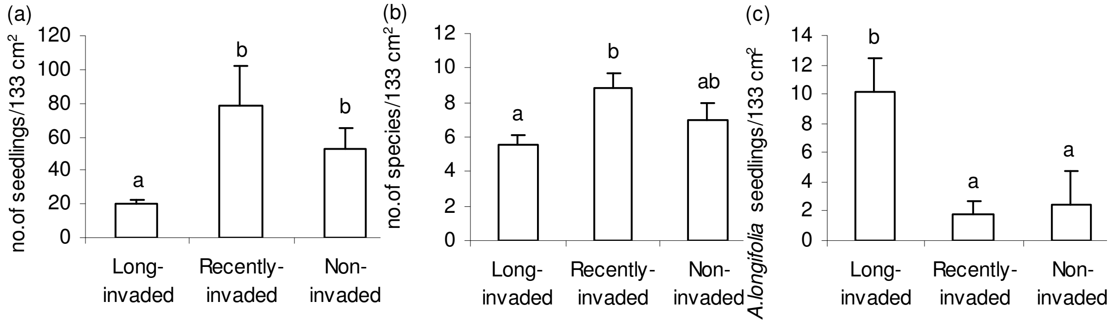

Figure 4.1 Seed bank density (a), species richness (b) and number of *A. longifolia* seedlings (c) (mean +SE, df = 4) in seed banks of areas long-invaded, recently invaded and non-invaded by *A. longifolia.* Bars with the same letters are not significantly different (LSD < 0.05). *Acacia longifolia* was not included in (a) and (b).

Effect of A. longifolia and litter removal on the seed banks - Although invasion age affected seedling density in long-invaded and recently invaded areas separately (see experiment 1), there was no between factors interaction ($F_{1,16} = 1.54$, $P = 0.233$) nor clearing treatment effect ($F_{1,16} = 0.004$, $P = 0.951$) (Figure 4.2a). There was a significant interaction between invasion age and treatments on species richness ($F_{1,16} = 8.42$, $P = 0.010$) with untreated plots (A) of recently invaded areas containing more species than all of the other plots (Figure 4.2b). The number of *A. longifolia* seedlings was affected by both the invasion age ($F_{1,16} = 15.08$, $P = 0.001$) and the treatments ($F_{1,16} = 5.56$, $P = 0.031$), with a nearly-significant interaction between these factors ($F_{1,16} = 3.77$, $P = 0.07$) (Figure 4.2c). The clearing treatment (ALR) in long-invaded areas markedly reduced the quantity of *A. longifolia* seedlings to levels similar to both plots (A and ALR) of recently invaded areas (Figure 4.2c).

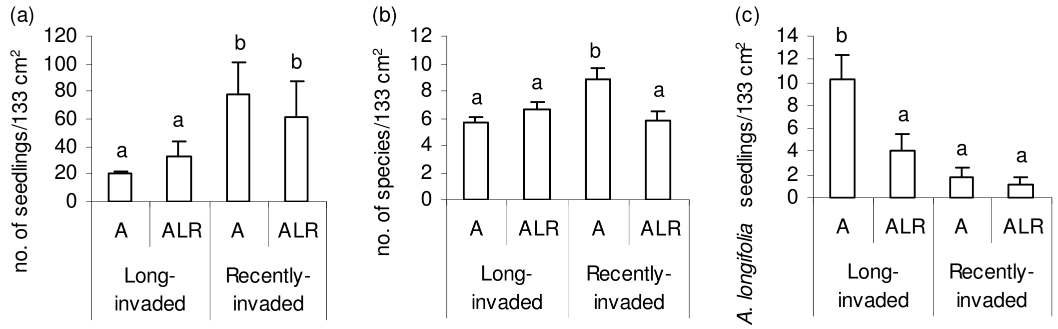

Figure 4.2 Seed bank density (a), species richness (b), and seedlings of *A. longifolia* (c) (mean +SE, df = 4) in seed banks of areas long-invaded and recently invaded by *A. longifolia,* in plots where both *A. longifolia* and litter were removed (ALR) and in untreated plots where *A. longifolia* was maintained (A). Bars with the same letters are not significantly different (LSD < 0.05). *Acacia longifolia* was not included in (a) and (b).

Seedlings diversity and similarity between seed banks

Seedlings diversity - In uncleared plots (A) diversity was significantly higher (p = 0.015) in recently invaded areas (2.21) than in long-invaded areas (2.01), but clearing (ALR) inverted the trend (recently invaded = 1.81, long-invaded = 2.06; p = 0.005). In long-invaded areas, clearing did not affect diversity (A = 2.01, ALR = 2.06; p = 0.34), while in recently invaded areas clearing caused a significant decrease in diversity (A = 2.21, ALR = 1.81; p < 0.0001). In recently invaded areas, clearing of *A. longifolia* and litter (ALR) resulted in diversity that was increasingly different from non-invaded areas (A = 2.21, non-invaded = 2.23, p = 0.43; ALR < non-invaded, p < 0.0001), while in long-invaded areas clearing resulted in diversity that was (barely) equal to non-invaded areas (A < non-invaded, p = 0.027; ALR = non-invaded, p = 0.062).

Similarity between seed banks - Similarity between invaded areas and non-invaded areas declined with length of *A. longifolia* invasion, with QS = 0.49 being recorded for the pairing of recently invaded untreated plots (A) and non-invaded plots, and decreasing to QS = 0.39 for the pairing of long-invaded untreated plots (A) and non-invaded areas. When *A. longifolia* plus litter were removed (ALR) the similarity between non-invaded species composition and invaded areas increased in both recently invaded areas (QS = 0.61) and long-invaded areas (QS = 0.55).

The similarity value (QS) of the species composition of seed banks was highest (0.67) between the untreated (A) and cleared plots (ALR) of long-invaded areas. In recently invaded areas, A and ALR were more dissimilar (QS = 0.57). The clearing treatments promoted similarity with QS rising from 0.44 for the comparison of uncleared plots (A) in recently invaded and long-invaded areas to 0.53 for the comparison of cleared plots (ALR) in the two areas.

Seed bank functional and other traits

Although seedling density, species richness and diversity were similar in recently invaded and non-invaded areas, the analyses of species traits and their relative abundances revealed substantial differences between these two areas (Figure 4.3). Even greater differences in trait attributes were noted when the seed banks of long-invaded areas were compared to those of recently invaded and non-invaded areas. In general, the clearing treatment (ALR) induced more changes in community structure in long-invaded areas than in recently invaded areas. Broadly, seed banks from non-invaded areas displayed a wider array and exhibited more even distribution of trait attributes than those from any of the invaded areas. The proportions of undetermined seedlings in the different areas varied between ca. 10% and 20%.

More than 30% of the seedlings that germinated in the samples from the untreated (A) long-invaded areas were *A. longifolia*. Recently invaded areas had the highest proportion of seedlings of other exotic and invasive species (Figure 4.3a). The exotic species detected in the seed banks were *Conyza* spp. and *Gamochaeta pensylvanica* (Willd.) Cabrera (very abundant particularly in recently invaded areas), *Carpobrotus edulis* (sporadic exclusively in long-invaded samples) and *Cortaderia selloana* (sporadic and only in recently invaded samples) (Appendix 4.2). Altogether, different classes of native species made up more than 65% of the seedlings that germinated in the samples from non-invaded areas, thus greatly exceeding the proportions observed in both invaded areas. The higher proportion of native species were seedlings from dune/generalist species.

Non-invaded areas showed the highest number of life forms (Figure 4.3b), only equalled by cleared plots (ALR) of long-invaded areas. The relative abundance of life forms was similar in both treatments of recently invaded areas, with therophytes comprising the vast majority of seedlings that germinated. In long-invaded areas the clearing treatment promoted greater changes in the proportions of life forms, with therophytes predominating in cleared plots (ALR), but being outnumbered by *A. longifolia* seedlings in the samples from untreated plots (A). Microphanerophytes (represented in the region by *Myrica faya* Aiton) only germinated rarely and exclusively in trays from untreated long-invaded areas (A). Chamaephytes, hemicryptophytes, cryptophytes and nanophanerophytes were vestigial in all areas.

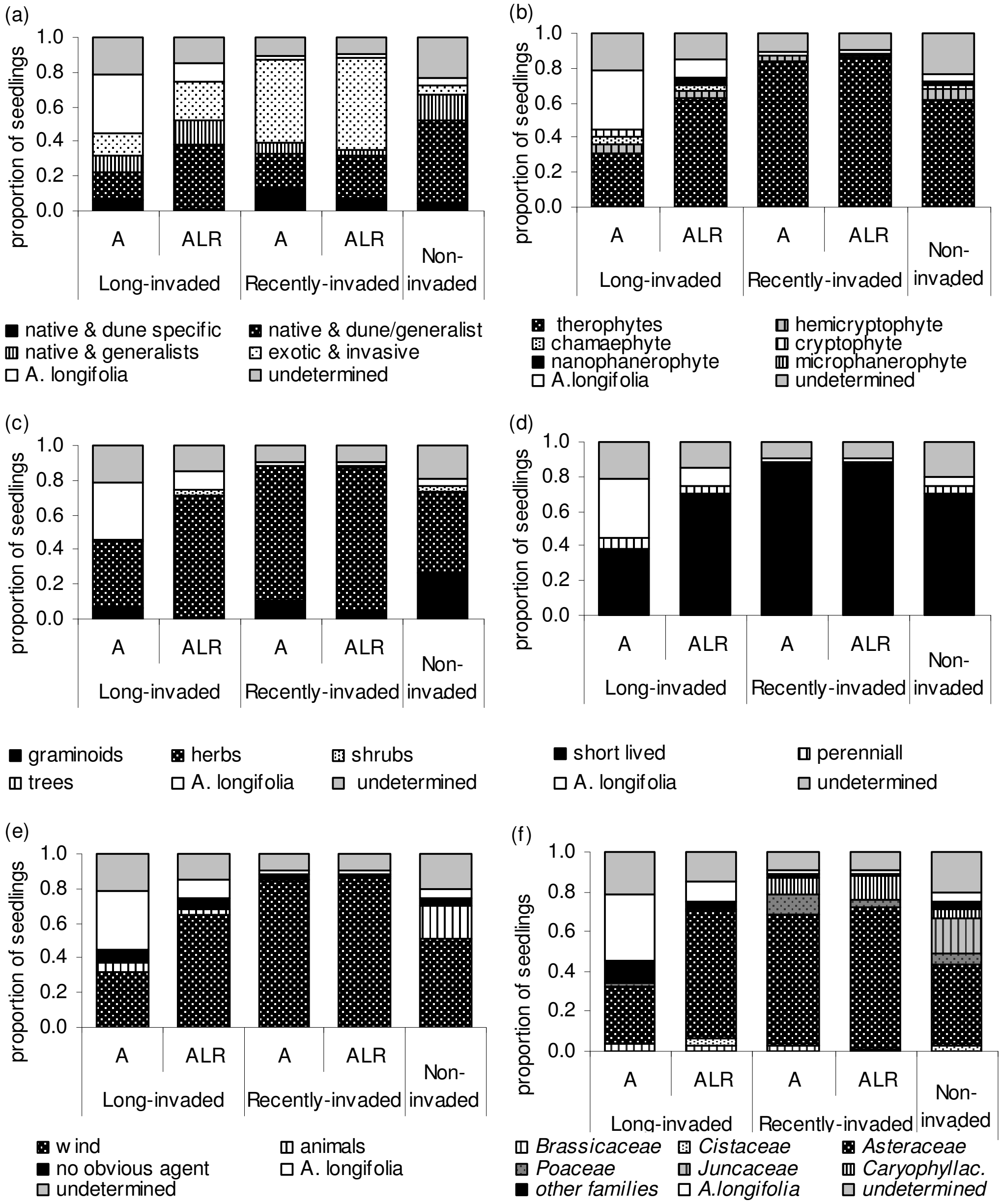

Figure 4.3 Relative abundance of emerged seedlings with each attribute of the plant traits (functional and other groups) analysed for seed banks of areas non-invaded, long-invaded and recently invaded by *A. longifolia,* in cleared (ALR) and untreated (A) areas: (a) species categories according to their origin & habitat; (b) life forms; (c) growth form; (d) longevity; (e) main dispersal agent; (f) taxonomic diversity according to families (families with proportionally less than 5% were grouped under "other families"; details in Appendix 4.2).

The seedlings that germinated in the samples from both invaded areas were mainly herbs (herbaceous dicotyledons), while graminoids and herbs were more evenly represented among the seeds that germinated in the samples from non-invaded areas (Figure 4.3c). In long-invaded areas the removal of both *A. longifolia* and litter (ALR) almost excluded graminoids. Shrubs and trees (except for *A. longifolia* itself) were poorly represented (no more than 1-2%) with shrubs occurring only in cleared plots (ALR) from both invaded areas and non-invaded areas and trees occurring only in untreated plots (A) from long-invaded areas.

Almost all the seedlings that germinated were short-lived species (Figure 4.3d) with the exception of those that germinated in the samples from untreated plots (A) of long-invaded areas, where *A. longifolia* (a perennial) was more abundant. Perennials were almost excluded from the seed banks of recently invaded areas. Seedlings of species dispersed by wind were dominant in almost all situations (Figure 4.3e). A greater proportion of seedlings in the samples from non-invaded areas were species that are dispersed by animals.

In terms of taxonomic diversity, *Asteraceae* was proportionally by far the most abundant family, being only outnumbered by *A. longifolia* in the untreated plots (A) from long-invaded areas (Figure 4.3f). The next most prominent taxa were *Poaceae* and *Caryophyllaceae* in recently invaded areas and *Juncaceae* in the non-invaded area. Seeds from almost all the other families were present in low numbers (rarely up to 1% of seedlings) and showed some differences between treatments and invasion ages (Appendix 4.2.) *Aizoaceae, Rubiaceae, Chenopodiaceae, Scrophulariaceae* and *Myricaceae* were only detected in long-invaded areas, the last being absent from cleared plots (ALR). *Primulaceae* and *Onagraceae* were only found in samples from recently invaded areas. *Fabaceae* (excluding *A. longifolia*) only occurred in the non-invaded areas.

DISCUSSION

Frequently-disturbed areas often show a close correlation between adult plants and soil seed bank species (Baptista & Shumway 1998). The system under investigation is highly disturbed by the invasion of an exotic species, which may have impacts that are different to other kinds of disturbance, and, to a lesser extent, by human pressure (tourism) and management practices (*e.g.* firebreaks and trails management). Our results show that the invaded seed banks at SJDNR do not clearly resemble either the quasi-mono-specific invaded system or the original interdune dune community, present before invasion (Neto 1993), although several species characteristic of the dunes persisted to invasion contributing to recovery of cleared areas (Sternberg *et al.* 2003).

Effects of invasion status on the seed bank

A resilient seed bank should include seed densities typical of the system, several species from several guilds, and a high proportion of native species. On the other hand, degraded seed banks have altered densities of seeds, few species or guilds and a high proportion of exotic invasive species (Frieswyk & Zedler 2006). Both invaded seed banks showed signs of severe degradation, being largely dominated by a few species (Bossuyt & Honnay 2008), namely exotic/invasive species. The exotics *Conyza* spp. and *Gamochaeta pensylvanica*, both short-lived species, clearly dominated the seed bank of recently invaded areas, despite not being particularly abundant in the extant vegetation (Marchante *et al.* 2004), while the long-invaded seed bank was dominated by the perennial *A. longifolia* accompanied by a few seeds of *Carpobrotus edulis*, also perennial. The higher longevity of the exotic/invasive species present in the seed banks of long-invaded areas indicates a more long lasting threat to these areas. Seeds of *Acacia* species, in particular, are known to have very high longevity (Cavanagh 1980; Daws *et al.* 2007), waiting in the soil for suitable conditions to germinate. On the other hand, the natural seed bank of non-invaded areas were more resilient, having a higher diversity of guilds, low abundances of exotic/invasive species and high proportions of native species, even though only a small proportion were dune specialists. However, the presence of *A. longifolia* seeds in areas not yet invaded showed that there is a risk of future invasion, especially after a fire event.

When subject to disturbance, seed bank densities of plant communities may show distinct changes, either increasing or decreasing (Pierce & Cowling 1991). In any case, the density of seeds in degraded communities is frequently distinct from that of corresponding unaltered communities (Frieswyk & Zedler 2006), which is corroborated by this study: both the decrease in the density of seeds in long-invaded areas and the increase in total seed density in recently invaded areas (mostly due to the exotic annuals referred to above) (Appendix 4.2) indicates degradation.

Measures of species richness, diversity, attributes of some plant traits, amount of *A. longifolia* seeds and similarity to non-invaded areas indicate that seed banks in long-invaded areas are more altered than those of recently invaded areas. For instance, *Caryophyllaceae* were absent and *Poaceae* were underrepresented in long-invaded seed banks, while both taxa were still well represented in recently invaded and non-invaded seed banks. The discrepancy could be due to the short-lived seeds which characterise several species of both these families. Studies have shown that *Cerastium diffusum* (*Caryophyllaceae*) (Mack 1976) and *Vulpia fasciculata* (*Poaceae*) (Watkinson 1978) do not accumulate long-lived soil seed banks in dunes. Long-invaded areas are therefore more impoverished of native species and are more likely to be reinvaded following manual clearing or fire. Similar trends have been found in areas invaded by other *Acacia* species in South Africa (Holmes & Cowling 1997a; Holmes 2002), although invasion ages were not always monitored in those studies.

In long-invaded areas, where *A. longifolia* had been present continuously for at least 20 years, the dense canopy and the thick litter layer has at least curtailed, if not prevented, the deposition of seeds from surrounding areas (*e.g.* from zoochorous species), as happens in other closed forest systems (Sayer 2006). Zoochorous plant species do not seem to cope well with *A. longifolia* invasion (Holmes & Cowling 1997b), being underrepresented in invaded areas while remaining frequent in non-invaded areas. In the recently invaded areas, movement of seeds (particularly from anemochorous species) into the area continued because the canopy of *A. longifolia* was more open (ca. 70%) and the litter layer was more sparse (1.43 ± 0.14 $Kg.m^{-2}$) than in the long-invaded areas (at least 80% *A. longifolia* cover and 2.05 ± 0.24 $Kg.m^{-2}$ of litter). The time of invasion would also affect seed bank traits because the viability of seeds (particularly small seeds) buried for long periods under thick litter layers diminishes (Eckstein & Donath 2005) along with the ability of viable seeds to germinate successfully in thick litter layers (Facelli & Pickett 1991). Noticeably, however, some dune -specific species, *e.g. Antirrhinum majus* L. *cirrhigerum* (Ficalho) Franco, with larger and longer-lived seeds (Shah *et al.* 2008) seem to have either overcome the barriers to immigration into thickets or survived the protracted periods of invasion, being exclusively detected (despite rarely) in long-invaded areas. Their absence in recently invaded areas could be due to the seeds being destroyed by the fire of 1995 and there not being enough time between the fire and sample acquisition for fresh seeds to spread into and accumulate in the invaded patches.

The high proportion of therophytes in the seed banks was expected as their establishment from seed is obligatory (Fenner & Thompson 2005), they are frequent in Portuguese dune ecosystems (Alves *et al.* 1998; Costa *et al.* 2000) and include many ‘opportunistic’ species, despite having limited conservation value (some are exotics). Being short-lived annual species that are relatively small, they are largely unable to compete with *A. longifolia* which is a large perennial species. Nevertheless, if therophytes create a dense cover over the soil surface, they may reduce germination of *A. longifolia* seeds and provide a transitional cover which will later be replaced by the less-numerous native perennial species in the seed bank. The seeds of perennial species are usually sparse in seed banks and these plants frequently have an ability to propagate from perennating buds located on storage structures as an alternative mean of reproduction (Sternberg *et al.* 2003). Some natural sand dunes lack persistent seed banks because conditions that induce dormancy (namely poorly aerated soils) are not always met (Owen *et al.* 2001). Additionally, recruitment of new individuals may have been limited (Thompson & Ceriani 2003) because some species require heat (Buhk & Hensen 2005), smoke (Reyes & Trabaud 2009), passage through animal intestines (Calviño-Cancela 2004) or other cues to induce germination and facilitate seedling emergence (Sternberg *et al.* 2003; Cottrell 2004). The lack of these types of cues in the glasshouse could partially explain why ca. 15% of the seedlings in this study (potentially some perennial species) did not survive to a stage where they could be identified.

Nevertheless, the lack of nanophanerophytes and chamaephytes (long-lived perennials, with many species characteristic of dunes, some with high conservation value), such as *Corema album* (L.) D. Don and *Cytisus grandiflorus* (Brot.) DC. (both endemic of the Iberian Peninsula), *Herniaria ciliolata* Melderis subsp. *robusta* Chaudhri and *Iberis procumbens* Lange subsp. *procumbens* (both endemic in Europe), *Crucianela maritima* L. and *Helichrysum italicum* (Roth) G. Don fil. subsp. *picardi* (Costa *et al.* 2000; Marchante *et al.* 2004; Silva 2006), and the very low numbers of other species, including *Cistus salvifolius* L. and *Ulex europaeus* L. subsp. *latebracteatus* (Mariz) Rothm, could also be an indication of a general degradation of seed banks in the studied dunes. Sampling size could also have contributed to the low levels of detection of these species. The deficiency of dune specialists, particularly in non-invaded areas, may be due to the fact that the selected non-invaded areas were not the most "typical" dune system, but rather the only places in the reserve that were located in dune situations that were equivalent to the invaded areas while being free of *A. longifolia* trees. In these areas graminoid species frequently predominate and apparently largely suppress germination of *A. longifolia* seeds. The graminoid species that were present (*Juncus* spp. and *Carex arenaria* L.) are frequently associated with relatively moist soils, which were also characteristic of some of the long-invaded areas where *A. longifolia* dominated. Species of *Juncus* and *Carex* produce very large amounts of small, long-lived seeds that are abundant in several communities (Bossuyt & Honnay 2008). The presence of these taxa may explain the higher density of seeds when compared to seed banks of other sand dunes systems (7-12 germinable seeds.m^{-2}) (Owen *et al.* 2001).

Bossuyt & Honnay (2008) analysed numerous community seed banks and support the notion that restoration from natural seed bank is only possible in areas degraded for less than five years. Our study corroborates this, at least partially, showing a decrease in the unaided recovery potential of the system as invasion age increases. Other studies in the SJDNR have shown that other parameters, namely microbiological and chemical properties of the soil in long-invaded stands of *A. longifolia* are also more altered and take more time to recover than in recently invaded stands (Marchante *et al.* 2009).

Effect of *A. longifolia* and litter removal on the seed banks

The effect of removing *A. longifolia* and the litter layer on species richness, diversity and amount of *A. longifolia* seeds varied with age of invasion. Removing *A. longifolia* and the litter layer resulted in greater similarity of the seed banks of long-invaded and recently invaded areas, indicating that an important part of the differences observed between invaded seed banks occurs among seeds that have accumulated over time in the litter layers.

Removing *A. longifolia* and the litter did not affect species diversity, species richness and seed bank density in long-invaded areas. However, litter removal decreased the number of *A. longifolia* seedlings, changed several trait spectra and left a seed bank whose species composition more closely resembled the seed banks of adjacent non-invaded areas, eventually creating a more-resilient seed bank (Frieswyk & Zedler 2006). Removing *A. longifolia* and litter resulted in a further decline of some taxa (*e.g. Cyperaceae, Rubiaceae, Myricaceae*) that had accumulated in the litter layer. Nevertheless, this loss was offset to some extent because other taxa appeared in the cleared areas. These were presumably recent additions which arrived during the short interval between clearing and collection of seed samples. Most of the plants in this group were herbaceous, short-lived, wind-dispersed species (particularly *Asteraceae*) which are frequently opportunistic and typical from early succession stages, and included both native generalists and exotics. More importantly, removing the litter decreased the number of *A. longifolia* seeds in long-invaded areas and thereby decreased the reinvasion potential of the invader. Indeed, moderate controlled fire could be used to eliminate the thick litter layer and simultaneously destroy many seeds of the invasive species (Richardson & Kluge 2008).

The number of species, particularly natives, and diversity declined in recently invaded areas when *A. longifolia* and the litter were removed, indicating that this type of clearing operation caused a further degradation of the seed bank (Frieswyk & Zedler 2006). Nevertheless, there was considerable similarity in the species composition of seed banks in recently invaded and non-invaded areas and clearing treatment did not induce changes in most plant traits. Invasion for a few years meant that the canopy was relatively open until recently so that seeds of other plant species continued to accumulate in the litter layer and the time interval was too short for natural attrition of the original seeds to have taken affect to any great extent. Removing the litter caused a decline or disappearance of some types of seeds, with at least 10 species being lost (Appendix 4.2), an outcome that would inadvertently contribute to a decline in the resilience of the system.

Although there are limitations in using seedling emergence to monitor species assemblages (Sternberg *et al.* 2003; Cottrell 2004), the method had benefits in this case because it: 1) allows an estimation of the available seed bank without major clearing efforts; 2) revealed species that were otherwise unseen in the invaded stands; and 3) indicated the invasion potential of *A. longifolia,* particularly in non-invaded areas. The results of this study indicated that due to higher impoverishment of the seed bank in long-invaded areas, recovery will probably be more dependent on the input of new seeds into cleared patches from neighbouring areas, a pattern that may not be easily predicted when sampling the resident seed bank. Due to low seed densities, patchiness of seed banks and short interval between clearing operations and sampling collection, it is not possible to confirm that seeds only found in cleared areas (ALR) had necessarily dispersed from neighbouring areas. Some may have germinated in trays due to litter removal and/or disturbance during the experimental manipulations. Increasing the sampling size could eventually contribute do detect more species.

CONCLUSIONS

As hypothesized, the results of this investigation showed that seed banks of areas invaded by *A. longifolia* for a longer period are more altered than the ones from recently invaded areas, suggesting that resilience of the system decreases as invasion time increases, namely because recovery of native plants from residual seed banks declines with time. The recovery of the coastal dunes is threatened by the overwhelming presence of *A. longifolia* and other aggressive invasive species in the seed bank. This situation becomes increasingly problematic with time because seeds of short-lived exotic species in recently invaded areas are replaced by seeds of perennial invader species in long-invaded areas. Regarding clearing treatment effects, there was a somewhat unexpected difference between invaded areas. The removal of *A. longifolia* trees and litter layer in long-invaded areas resulted in few species being lost and higher similarity with native areas, including functional trait spectra, which may further enhance recovery of the system. In recently invaded areas, on the contrary, the removal of litter contributed to a further degradation of the seed bank, with many species being lost and a decrease in diversity when compared with areas where *A. longifolia* remained. The results also show that the assessment of traits other than species richness, abundance and diversity of the soil seed bank revealed patterns that would otherwise have been unnoticed.

From a management point of view, if invaded areas are left intact, more vigorous and probably more expensive management efforts will then be needed if the invaded systems are to be restored. A strategy to control or manage *A. longifolia* needs to include dealing with reinvasion by seedlings and sustaining the recovery of native communities, mainly in the long-invaded areas where the removal of the litter layer was an effective mechanism for decreasing the amount of *A. longifolia* seeds in the system. Although many native species are present including several species characteristic of the dunes, which is positive, many others are missing which, especially when summed with exotics, is negative and needs to be considered when planning recovery strategies. Seed banks alone do not seem to be sufficient to allow full system recovery after clearing. Chapter 5 evaluates the recovery of vegetation after the removal of trees with or without the litter layer, including the time factor, which further assists in the validation of the present approach.

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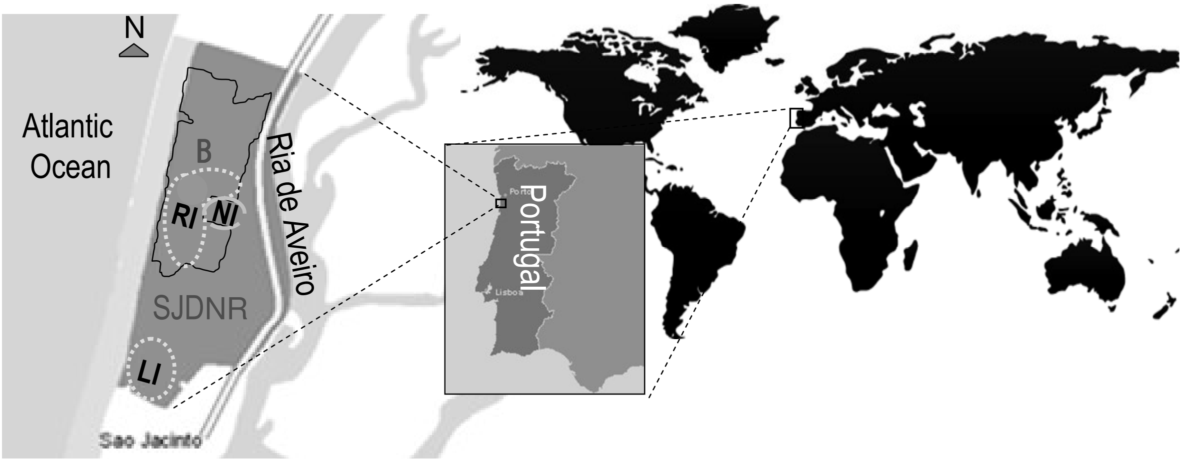
Appendix 4. 1



Location of the study area, São Jacinto Dunes Nature Reserve (SJDNR), in central-northern coast of Portugal (adapted from http://www.multimap.com/), showing areas where experiments of soil seed bank took place. RI - recently invaded areas; LI - long-invaded areas: NI – non-invaded areas; B, area burnt in 2005, and largely invaded after that (recently invaded areas).

Appendix 4.2

Total seedling density of each species in seed banks of areas non-invaded, long-invaded and recently invaded by *A. longifolia,* at untreated areas (A) and cleared areas with removal of both *A. longifolia* and litter (ALR). 6623 cm^3 of soil were collected from a surface area of 662 cm^2 within each treatment.

Family	Raunkiær life form		Species	Long-Invaded		Recently - Invaded		Non-
				A	ALR	A	ALR	Invaded
Aizoaceae	chamaephyte	e	*Carpobrotus edulis* (L.) N.E.Br.	2	1	--	--	--
Asteraceae	hemicryptophyte	n	*Andryala integrifolia* L.	6	5	8	--	9
	therophyte	e	*Conyza* spp.	16	6	83	95	12
	therophyte	e	*Gamochaeta pensylvanica* (Willd.) Cabrera	2	34	110	71	--
	hemicryptophyte	n	*Hypochaeris glabra* L.	--	--	1	--	--
	therophyte	n	*Logfia minima* (Sm.) Dumort	1	1	--	1	87
	hemicryptophyte	n	*Picris echioides* L.	--	3	--	--	--
	therophyte	n	*Pseudognaphalium luteum album* (L.)Hilliard &B.L.Burtt	15	52	40	57	5
	therophyte	n	*Senecio vulgaris* L.	3	5	10	--	--
	therophyte	n	*Sonchus oleraceus* L.	--	13	12	1	1
Brassicaceae	therophyte	n	*Cardamine hirsuta* L.	6	5	9	--	--
	hemicryptophyte	n	*Hirschfeldia incana* (L.) Lagrèze-Fossat	--	--	2	--	--
Caryophyllaceae	therophyte	n	*Cerastium diffusum* Pers. *diffusum*	--	--	1	7	--
	therophyte	n	*Polycarpon tetraphyllum* (L.) L.	--	--	8	16	12
	therophyte	n	*Sagina apetala* Ard. *erecta* (Hornem.) F.Hermann	--	--	27	14	--
	therophyte	n	*Silene gallica* L.	--	--	1	--	--
Chenopodiaceae	therophyte	n	*Chenopodium album* L.	1	--	--	--	--
Cistaceae	nanophanerophyte	n	*Cistus salvifolius* L.	--	7	--	3	3
	therophyte	n	*Tuberaria guttata* (L.) Fourr.	--	--	1	--	4
Cyperaceae	cryptophyte	n	*Carex arenaria* L.	5	--	1	--	5
Fabaceae	microphanerophyte	e	*Acacia longifolia* (Andrews) Willd	50	20	8	6	12
	nanophanerophyte	n	*Ulex europaeus* L. *latebracteatus* (Mariz) Rothm	--	--	--	--	4
Juncaceae	therophyte	n	*Juncus bufonius* L.	--	--	--	--	21
	therophyte	n	*Juncus capitatus* Weigel.	--	--	--	1	16
	therophyte	n	*Juncus pygmaeus* L. C. M. Richard in Thuill.	--	--	--	--	12
	undetermined	n	*Juncus* sp.	--	--	1	--	--
Myricaceae	microphanerophyte	n *	*Myrica faya* Aiton	1	--	--	--	--
Onagraceae	hemicryptophyte	n	*Epilobium tetragonum* L.	--	--	1	--	--
Poaceae	hemicryptohphyte	n	*Agrostis stolonifera* L.*pseudopungens* (Lange) Kerguélen	--	--	--	--	6
	therophyte	n	*Aira praecox* L.	3	--	21	9	--
	therophyte	n	*Briza maxima* L.	--	--	1	--	--
	hemicryptophyte	e	*Cortaderia selloana* (Schultes) Asch. & Graebner	--	--	2	2	2
	hemicryptophyte	n	*Dactylis glomerata* L. subsp. *hispanica* (Roth.) Nyman	1	--	--	--	--
	therophyte	n	*Vulpia alopecuros* (Schousboe) Dumort. *alopecuros*	--	--	4	--	--
	therophyte	n	*Vulpia membranaceae* (L.) Dumort.	--	--	8	--	--
	undetermined		*Poaceae*	--	1	5	--	7
Primulaceae	chamaephyte	n	*Anagallis arvensis* L.	--	--	1	--	--
	therophyte	n	*Asterolinum linum-stellatum* (L.) Duby in DC	--	--	3	1	--
Rubiaceae	therophyte	n	*Galium minutulum* Jordan	1	--	--	--	--
Scrophulariaceae	chamaephyte	n	*Antirrhinum majus* L. *cirrhigerum* (Ficalho) Franco	1	1	--	--	--
Solanaceae	chamaephyte	n	*Solanum nigrum* L. subsp. *nigrum*	5	5	1	1	1
Thyphaceae	cryptophyte	n	*Typha latifolia* L.	2	--	--	--	--
			undetermined	32	27	37	30	57
			total number of species per invasion status / age	***18***	***15***	***27***	***15***	***18***
			total seedling abundance per invasion status / age	***147***	***186***	***398***	***315***	***276***

e/n: n = native species; e = exotic species

* considered to be exotic by some authors

Chapter 5

Post-clearing recovery of coastal dunes invaded by *Acacia longifolia*: is duration of invasion relevant for management success?

ABSTRACT

1. Despite having the highest priority for conservation by IUCN, the structure and function of many coastal dunes are threatened by invasive plant species. In Portuguese dunes, *Acacia longifolia* is one of the most prominent invasive species. Removal of invasive plants is crucial for preventing loss of biodiversity but the likelihood that restoration efforts will succeed should be assessed before embarking in expensive management plans. However, post-clearing studies are scarce, usually short-term and rarely consider duration of invasion.

2. Recovery of plant communities was monitored over six years in a coastal dune after removal of *A. longifolia* either with or without the associated litter layer. Duration of the invasion period before clearing was considered. Species richness, plant cover, diversity, evenness, species traits and turnover rates were analysed.

3. Duration of invasion was the main determinant of resilience of invaded systems. Recently invaded areas had higher species richness, plant cover, initial diversity and turnover rates than long-invaded areas. The post-clearing flora of both areas was dominated by generalist native species which, over time, were increasingly replaced by species more adapted to dunes. However, six years after removal of *A. longifolia* several species typical of dunes were still missing. Exotic species were more abundant in recently invaded areas. Therophytes were the most abundant life form immediately after clearing but nanophanerophytes, chamaephytes and *A. longifolia* increased with time.

4. Seedlings of *A. longifolia* were more abundant in cleared long-invaded areas. Effects of litter removal were most evident in cleared long-invaded areas, promoting increased species richness and plant cover, and decreasing susceptibility to reinvasion.

Synthesis and applications: Sand dunes densely invaded by a woody species have some inherent resilience but, as the invasion periods extends, recovery of the original communities is increasingly less likely and the probability of reinvasion increases. Therefore, more-recently invaded areas should be prioritized for clearing. When invasion is accompanied by an accumulation of litter, this should be removed to enhance recovery of native flora. In most situations, restoration needs to be supported by active management actions (*e.g.* litter removal, transplantations, fire); to establish the original ecosystem may be an unrealistic restoration target.

Keywords: ecosystem resilience, *Fabaceae/Leguminosae*, Sydney golden wattle, long-term monitoring post-clearing, invasion time, experimental litter removal, reinvasion post-clearing.

INTRODUCTION

Invasive plant species are considered one of the primary threats to biodiversity (Richardson *et al.*, 2000; Richardson *et al.*, 2008; Gaertner *et al.*, 2009) as well as to the integrity and function of ecosystems (Vitousek, 1990; Blossey, 1999; Marchante *et al.*, 2008a). Ever-escalating problems caused by invasive species increasingly require intervention for their removal and the restoration of natural habitats. Before embarking on expensive management programs, the probability that restoration efforts will succeed should be assessed, including the possibility that the invasive species will reinvade after its initial removal (Zavaleta, Hobbs & Mooney, 2001). Long-term monitoring after control operations can provide valuable ecological information by revealing how changes in the abundance of species influences properties and processes of ecosystems, which in turn helps to guide management decisions (Blossey, 1999). Despite these real benefits, most studies on the effects of invasive plants removal are of short duration and/or frequently fail to include effects on native plant species (Ogden & Rejmánek, 2005; Hejda & Pyšek, 2006).

Coastal ecosystems are particularly vulnerable to disturbance and have been given the highest priority for conservation action by IUCN (Shine, Williams & Gündling, 2000). Coastal dune ecosystems are not only fundamental barriers against the advance of the ocean (Carter, 1995), they also harbour endemic plant species and form unique habitats (Honrado *et al.*, 2006). The stability and natural dynamics of coastal dunes relies on natural plant communities which stabilise the labile sands and minimize the effects of erosion (van der Putten & Peters, 1995).

Along the Portuguese coast, pristine dune systems are becoming increasingly rare, with native plant species being replaced by several invasive exotic species, including *Acacia longifolia* (Andrews) Willd (Alves *et al.*, 1998; Marchante, Marchante & Freitas, 2003), *A. saligna* (Labill.) H.L. Wendl. (Marchante, 2001), *Carpobrotus edulis* (L.) N. E. Br. (Campelo, 2000) and *Cortaderia selloana* (Schultes) Asch. & Graebner (Marchante, Freitas & Marchante, 2008). *Acacia longifolia* (long-leafed wattle) (*Fabaceae/ Leguminosae*), the most prominent and widespread invader in Portuguese dunes, was introduced in early 20th century to curb sand erosion. Besides deliberate planting, its abundance and distribution has increased greatly following fire events with major impacts that escalate with time (Marchante *et al.*, 2008b; chapter 2). In common with other *Acacia* species, *A. longifolia* is a nitrogen fixing tree (Rodríguez-Echeverría, Crisóstomo & Freitas, 2007) that produces large quantities of slowly-decomposing litter (Pereira, Graca & Molles, 1998; Marchante, 2008) which accumulates in deep layers beneath the almost mono-specific, dense stands in dune areas which are otherwise almost litter deprived.

Plant litter and its decomposition are considered a vital part of ecosystem functioning, increasingly influencing vegetation structure as litter accumulates (Xiong & Nilsson, 1999). Its effects vary among ecosystems and depend on litter composition and quantity, with repercussion for species richness and above-ground biomass (Facelli & Pickett, 1991; Sayer, 2006). In general, litter suppresses germination of small seeds and seedling establishment, while germination of large seeds and seedling establishment are facilitated (Sayer, 2006). Suppression occurs because litter forms a barrier which prevents seeds from reaching the soil. Small seeds that germinate in the litter layer fail either because their roots do not reach the soil, or their shoots are unable to reach the surface (Facelli & Pickett, 1991). In large-seeded species survival may be facilitated because the seeds are less likely to be retained by the litter and input of nutrients from the decomposing litter may nurture seedlings while the litter protects the seeds and seedlings from predators (Sayer, 2006). The potential for major disruption of ecosystems is most evident when invasive species that produce large quantities of litter invade ecosystems that naturally have less litter. In such cases, the ever increasing quantities of litter result in abnormal accumulations of organic matter which persists because there is insufficient or inappropriate soil fauna and microbiota to decompose it properly.

Considering that plant communities are dynamic through time, evaluation of recovery of ecosystems after removal of invasive plants requires median to long-term monitoring. However, most of these types of studies have been too short to provide comprehensive results (Maron & Jefferies, 2001; Berlow, D'Antonio & Swartz, 2003; Ogden & Rejmánek, 2005; Hejda & Pyšek, 2006). Furthermore, studies which consider duration of invasion prior to clearing are very scarce (Strayer *et al.*, 2006). Against this background, we carried out a study to evaluate the resurgence of native and exotic plant species over a six year period in a coastal dune ecosystem dominated by *A. longifolia* for different durations, predicting that the resilience of communities of native species will decrease as invasion time increases and that removal of litter along with the invasive plants will facilitate recovery of native plant communities. The experimental set up was established in order to test management solutions and offer concrete recommendation to managers dealing with invasive species.

METHODOLOGY

Study site - The study area was located in the São Jacinto Dunes Nature Reserve (hereafter SJDNR) which is located on the central-northern coast of Portugal (40º 39’ N, 8º 44’ W). SJDNR covers about 660 ha and is bordered by the Atlantic Ocean to the West and by the Ria de Aveiro estuary to the East. The climate is Mediterranean with Atlantic influence. The mean annual precipitation is 920 mm and mean monthly temperatures range from 10.2ºC in January to 20.2ºC in June. Dominant winds blow from the Northwest and North, approaching from the Ocean. Historically, the area supported open vegetation characterized by several communities dominated by shrub and herb species and sporadic small trees (Neto, 1993; Honrado *et al.*, 2006).

Acacia longifolia, which was introduced into SJDNR early in the 20th century to curb movement of sand, has subsequently proliferated and extensively invaded much of the reserve (Marchante *et al.*, 2008b). When this study took place, *A. longifolia* was dominant over 150 ha and occurred in mixed association with other plant species over 200 ha (Guimarães, 2004). Other exotic species such as *Carpobrotus edulis* and *Cortaderia selloana* were also invasive in the area but to a lesser extent. The invaded portions of SJDNR can be divided into long-invaded and recently invaded areas. Long-invaded areas have had *A. longifolia* for several decades and are largely restricted to a small portion along the South border of the SJDNR. The recently invaded areas came about when *A. longifolia* proliferated over a large (previously-unaffected) area of the reserve after a natural fire which destroyed about 200 ha of vegetation, including pine plantations, during the 1995 summer (Silva, 1997). Before the fire, the burnt areas had low numbers of *A. longifolia* plants, mostly in the under-story of *Pinus pinaster* Aiton. The mono-specific, arboreal stands of *A. longifolia* had caused significant changes in community structure (Marchante *et al.* 2003) and ecosystem functioning (Marchante *et al.* 2008b, 2008c), including deposition of large quantities of leaf litter (Marchante *et al.*, 2008b), reduction of light at soil level and replacement of diverse herbaceous and shrubs communities (Neto, 1993; Honrado *et al.*, 2006; chapter 2).

Experimental design - In each of the two invaded areas (long and recently invaded), a complete randomized block design was used to define five blocks, each consisting of three 100 m^2 plots with similar *A. longifolia* cover. One of three treatments was randomly assigned to one plot in each block. The treatments were: 1) plots cleared of *A. longifolia* (abbreviated AR) by cutting the trees with chainsaws at ground level; 2) plots where *A. longifolia* trees were removed and the litter layer was also removed (ALR); and 3) plots with both *A. longifolia* and litter left intact as untreated controls (A). After treatments had been applied according to the experimental design, conditions in cleared plots (AR and ALR) were distinctly different from untreated plots (A) (Table 5.1).

After trees and litter were removed in October 2002, two 2 x 10 m transects were demarcated to record plant species growing in each plot. Transects were monitored twice a year (late January/early February and May), from December 2002 to May 2004, and then once every 2 years until May 2008. Parameters measured were: 1) plant species present, 2) species cover, 3) number of *A. longifolia* seedlings and 4) soil litter coverage.

Table 5.1 Characterization of experimental plots, after establishment, in areas long-invaded and recently invaded by *Acacia longifolia.*

		A. longifolia maintained (A)	*A. longifolia* removed (AR)	*A. longifolia* + litter removed (ALR)	Source
A. longifolia cover (%)	Recently-invaded	Ca. 70%	--	--	(1)
	Long-invaded	> 80%	--	--	
Leaf litter (Kg.m^{-2})	Recently-invaded	1.43 ± 0.14		--	(2)
	Long-invaded	2.05 ± 0.24		--	
Light intensity (at soil level) (μmol.m^{-2}.S^{-1})	Recently-invaded	283.6 ± 61.4	1093.5 ± 127.1		
	Long-invaded	170.2 ± 41.0	1061.1 ± 73.9		

(1) Chapter 2; (2) Marchante *et al.* (2008b).

Data analysis - *Recovery of plant communities post-clearing*: recovery of communities was first characterized using measures of species richness, plant cover, Shannon diversity and evenness separately. Because *A. longifolia* was the potential driver of change in the plant community, it was excluded from the analysis of species richness and total plant cover. Pielou evenness index J (from 0 to 1) and Shannon diversity index H' (from 0 to ≈ 5, but usually found to fall between 1.5 and 3.5) calculations included *A. longifolia* as these indexes reflect the presence of a dominant species (Magurran, 1988). All parameters were analyzed by repeated-measures MANOVA with time post-clearing as a within subject factor and invasion status and treatment as between-groups factors.

Species traits and turnover rate in plant communities post-clearing: the species were categorized into biological and ecological attributes which were classified under traits (Table 5.2).

Table 5.2 Plant traits and their respective attributes used to classify the species recorded in the study plots

Trait	Attribute	Source
Biological trait: Raunkiær life forms *	Therophyte, hemicryptophyte, chamaephyte, geophyte, nanophanerophyte, microphanerophyte	1, 2
Ecological trait: Species biogeographic distribution	Exotic & invasive, native & generalist (species that occur in several different habitats), native & dune specialist (species that are limited to dunes), native & dune/generalist (species that occur in dunes and other habitats but are limited to sandy soils)	2, 3

1 = Field observations; 2 = Franco & Afonso (1971-2003); 3 = Marchante, Freitas & Marchante (2008).

* In species with more than one attribute, the attribute dominant in the studied system was considered.

For each treatment, the area covered by species with a particular attribute was summed and divided by the total area covered by all the species within the trait, to produce a relative abundance of each attribute per treatment. *Acacia longifolia* was treated separately.

Species turnover rate (TR) was calculated to measure species shifts between May 2003 and 2008, as follows: TR =0.5 (L+G), where L is the number of species lost and G is the species gained during a defined period (Hilli, Kuitunen & Suhonen, 2007). Turnover rates differences were analysed with a 2-way factorial ANOVA. The time interval represents the changes observed in the full study period, with measurement made each year in the same season being compared to avoid seasonal effects as a source of discrepancy.

Susceptibility to (re)invasion post-clearing was based on: a) number of *A. longifolia* seedlings; and b) *A. longifolia* cover (saplings and trees), which were analyzed by repeated-measures MANOVA with time post-clearing as a within subject factor and invasion status and treatment as between-groups factors.

In all statistical analyses, mean differences were separated with LSD test at 5% level of significance. STATISTICA 6.0 (StatSoft, Inc., 2001, http://www.statsoft.com) was used.

RESULTS

Recovery of plant communities post-clearing Over the six year monitoring period, 83 species of vascular plants in 26 families were identified in the survey plots; six were exotics and 77 natives. There were 77 species in recently invaded areas as opposed to 56 species in long-invaded areas. More species were found in plots where both *A. longifolia* and litter were removed (ALR) (74 species and 49 species in recently- and long-invaded plots, respectively) than in cleared areas where only *A. longifolia* was removed (AR) (64 species and 48 species in recently- and long-invaded plots, respectively). In untreated areas (A) there were approximately half as many species (36 species and 26 species in recently- and long-invaded plots, respectively) as in the cleared areas (Appendix 5.1).

A significant interaction between invasion age, clearing treatments and time post-clearing for species cover ($F_{12, 60} = 10.82$, $P < 0.0001$) and species richness ($F_{12, 60} = 2.67$, $P = 0.006$) was verified. In the first two months after clearing, neither plant cover (Figure 5.1) nor species richness (Figure 5.2) showed clear patterns of change, with some exceptions, *e.g.* by December 2002, in plots in recently invaded areas where only *A. longifolia* had been removed (AR), species richness was higher than in all the other plots. There was a significant increase of species cover (Figure 5.1) and richness (Figure 5.2) in both cleared plots (AR and ALR), that ranged from ca. 20% cover and four species immediately after clearing, to more than 80% cover and 12 species by May 2006. Generally, in untreated plots (A) plant cover was sparser and species richness was lower than in cleared plots (AR and ALR) of both invaded areas; both of these parameters showed little change over time.

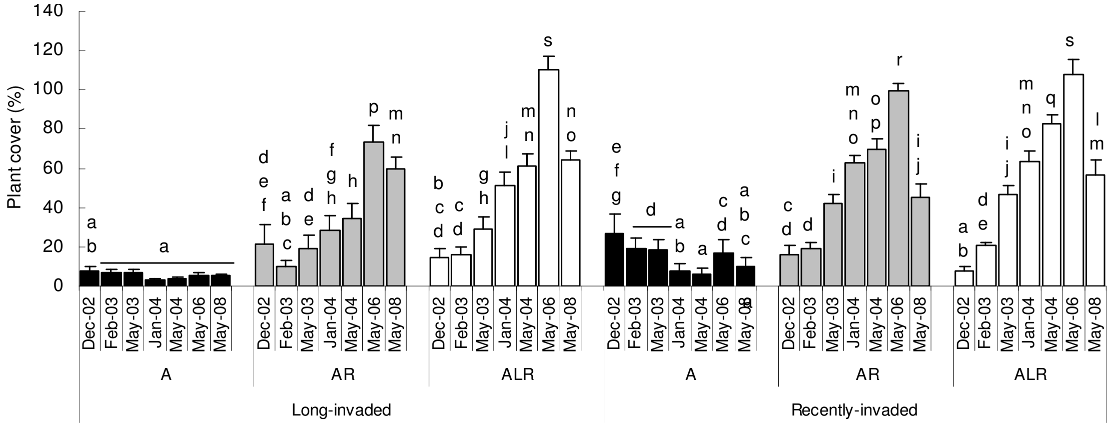


Figure 5.1 Changes in plant cover (mean +SE; n = 10) over time after clearing treatments in areas long-invaded and recently invaded by *Acacia longifolia*: AR - *A. longifolia* removed, ALR - both *A. longifolia* and litter layer removed, and A - *A. longifolia* maintained. Different letters above bars indicate statistically significant differences at P < 0.05 (LSD test). Values do not include *A. longifolia* cover.

Plant cover was lower in long-invaded areas compared to recently invaded areas. With time, plant cover in plots where the litter layer was removed (ALR) became significantly higher than in plots where the litter layer was left intact (AR). On the last sampling occasion there was a significant reduction in plant cover (Figure 5.1).

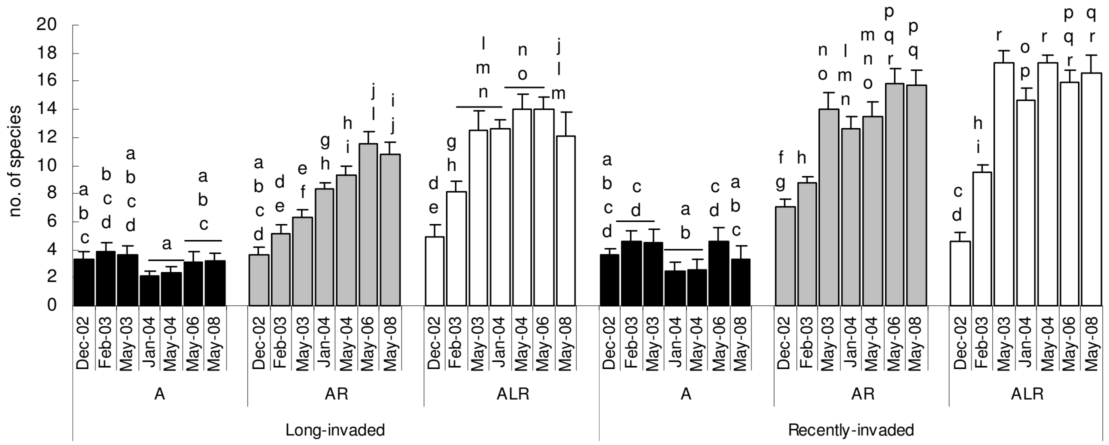


Figure 5.2 Changes in species richness (mean +SE; n = 10) over time after clearing treatments in areas long-invaded and recently invaded by *Acacia longifolia.* Different letters above bars indicate statistically significant differences at P < 0.05 (LSD test). Values do not include *A. longifolia.* Abbreviations as for Figure 5.1.

After clearing, regardless of litter removal (ALR) or not (AR), less species appeared in long-invaded areas (12-14 species/plot) than in recently invaded areas (16-18 species/plot) (Figure 5.2). In long-invaded areas, species richness was higher in plots where the litter was removed (ALR) than in plots where it was left (AR). In recently invaded areas the species richness in the plots was generally similar under the two clearing treatments.

Shannon's diversity and Pielou's evenness were significantly lower in untreated plots (A) than in cleared plots regardless of litter being removed (ALR) or not (AR) (Figure 5.3). By May 2003, both Shannon diversity and evenness of untreated plots (A) were significantly higher in recently invaded areas than in long-invaded areas. Five years later there was no difference. Soon after clearing (May 2003) plant diversity was lower in plots where *A. longifolia* was removed alone (AR) than in plots where the plants and litter were removed (ALR) (Figure 5.3a), but five years latter diversity had significantly decreased in ALR and become similar to AR. Evenness was similar between treatments, and in general decreased with time (Figure 5.3b)

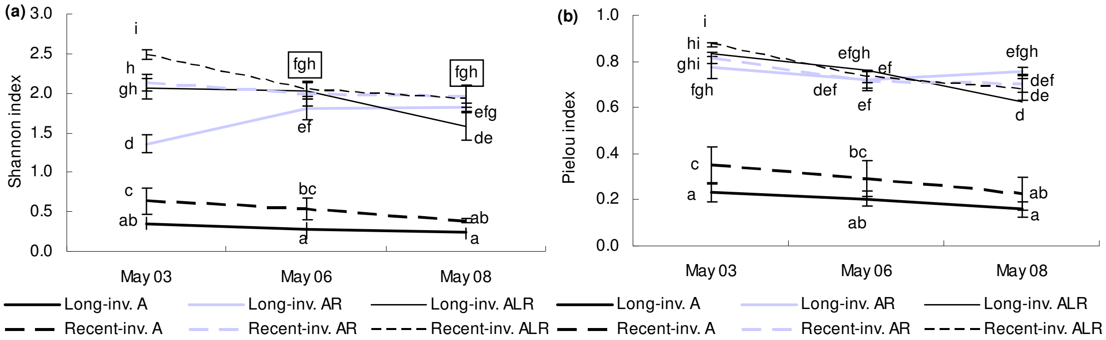


Figure 5.3 Diversity measures over time after clearing treatments in areas long- and recently invaded by *Acacia longifolia.* (a) Shannon Diversity and (b**)** Pielou's Evenness. Different letters in the lines indicate statistically significant differences at $P < 0.05$ (LSD test). Indexes were calculated with *A. longifolia* included. Abbreviations as for Figure 5.1.

Species traits and turnover rate in plant communities after clearing. Analyses of species traits showed more changes in cleared plots (AR and ALR) than in untreated plots (A) (Figure 5.4). In the early stages of recovery, therophytes were predominant in cleared plots. Although their relative abundance declined with time, they remained the most abundant group in all the plots except those without litter (ALR) in long-invaded areas where nanophanerophytes became dominant (Figure 5.4a). The proportions of chamaephytes, nanophanerophytes and *A. longifolia* increased with time. Several life forms were absent, or rare (relative abundance less than 1%), in untreated plots (A) of recently- and long-invaded areas.

The majority of species that appeared in cleared plots were natives (Figure 5.4b) accounting for over 70% of the cover. In untreated plots (A) *A. longifolia* dominated without any major changes over time, while in cleared plots it increased continuously.

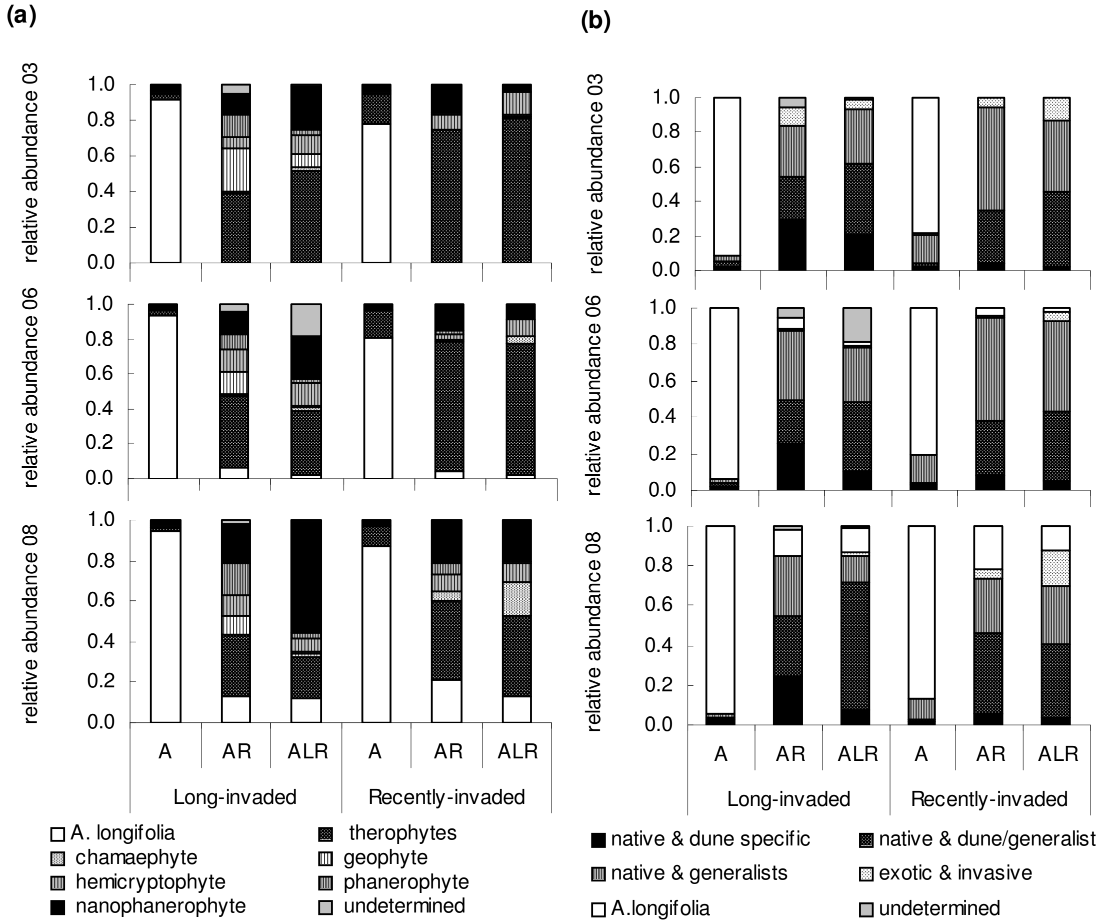


Figure 5.4 Differences in relative abundance of species traits among invasion age and treatments, in May 2003, 2006 and 2008. The species were categorized according to (a) a biological trait: Raunkiær life forms, and (b) an ecological trait: species biogeographic distribution. Abbreviations as for Figure 5.1.

The most abundant species in all of the cleared plots were generalists, including species associated with both dunes and other sandy habitats (dune/generalists) as well as others associated with a wider range of habitats (generalist). In the early phase of recovery, the wide-ranging generalist species were prevalent but decreased with time, while dune/ generalists increased with time, especially in long-invaded areas. Several exotic, invasive species were detected in cleared plots of both areas by May 2003, their abundance increasing proportionately with time in recently invaded areas, especially in cleared plots without litter (ALR). The relative abundance of species typical from dunes was highest in cleared plots of long-invaded areas, particularly when only *A. longifolia* was removed (AR).

Species turnover was affected by invasion time ($F_{1, 54} = 9.99$, $P = 0.003$) and clearing treatments ($F_{2, 54} = 24.26$, $P < 0.001$) with no interaction between factors ($F_{2, 54} = 0.89$, $P = 0.418$). In the 5 year interval following clearing, recently invaded areas showed significantly ($p = 0.003$) more alteration of species (TR = 5.5 ± 0.5) than long-invaded areas (TR = 3.9 ± 0.4). Species turnover was much higher in cleared areas (AR = 5.7 ± 0.6 and ALR = 6.1 ± 0.4) than in untreated ones (A = 2.2 ± 0.3) (A *vs.* AR and A *vs.* ALR, $p < 0.001$). Litter removal did not significantly affect species turnover (AR *vs.* ALR, $p = 0.522$).

Susceptibility to (re)invasion post-clearing. There was a significant interaction between invasion age, clearing treatments and time after clearing with respect to reinvasion after clearing, based on both the total number of *A. longifolia* seedlings ($F_{12,60} = 15.31$, $P < 0.0001$) and numbers that survived to become saplings and trees ($F_{12,60} = 2.08$, $P = 0.032$). Counts of *A. longifolia* seedlings revealed that a high number of viable seeds had accumulated in the soil, with particularly high peaks of germination in cleared plots (both AR and ALR) of long-invaded areas (Figure 5.5). Germination of *A. longifolia* seedlings was heterogeneous (e.g., ranged from 4 to 165 seedlings m^{-2} in ALR plots of long-invaded areas, in January 2004). Six years after clearing there were still seeds germinating. Many of the seedlings did not survive from one monitoring period to the next but throughout the study new seedlings were found on every sampling occasion including the last.

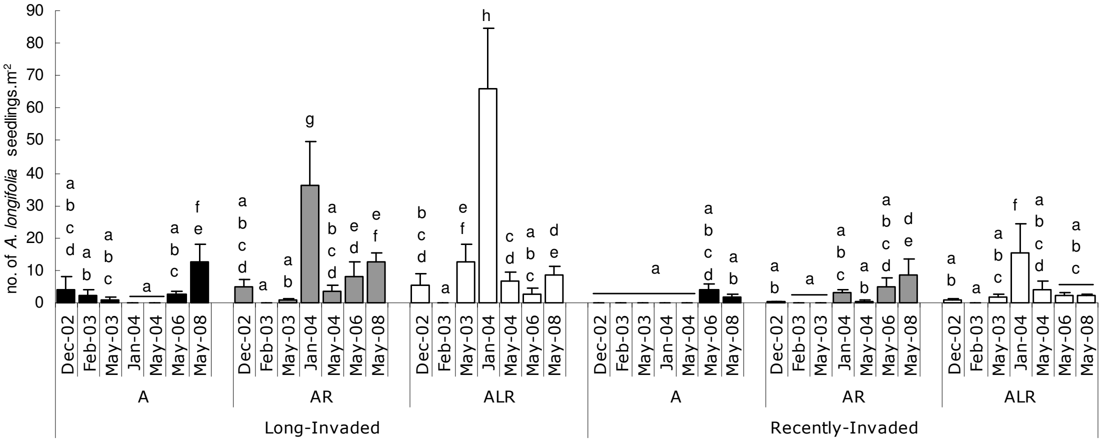


Figure 5.5 Germination of *Acacia longifolia* seedlings (mean + SE m^{-2}; n = 10) over time in long-invaded and recently invaded areas after clearing treatments. Different letters above bars indicate statistically significant differences at $P < 0.05$ (LSD test). Abbreviations as for Figure 5.1.

On average, cover of *A. longifolia* in untreated plots (A) was 10% higher in long-invaded areas than in recently invaded areas (Figure 5.6). The successful reinvasion by *A. longifolia* (recorded as seedlings that grow to saplings and trees) occurred progressively. By May 2008 there was a significant increase of the invasive species cover in all cleared plots (AR and ALR). About 6 years after clearing, there was a higher cover of *A. longifolia* in cleared plots where litter had been left (AR) than in plots where litter was removed (ALR).

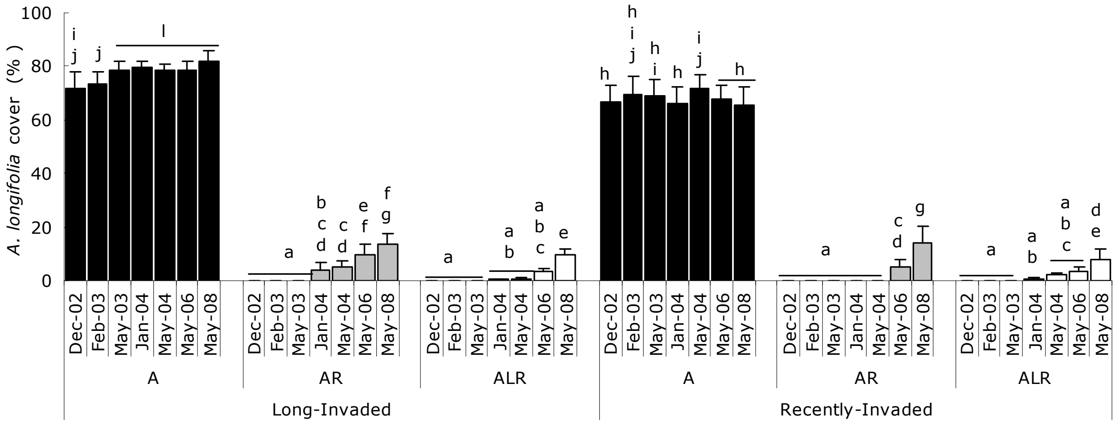


Figure 5.6 Percentage cover by *A. longifolia* saplings and young trees over time (mean +SE; n = 10) in long-invaded and recently invaded areas after clearing treatments. Different letters above bars indicate statistically significant differences at P < 0.05 (LSD test). Abbreviations as for Figure 5.1.

DISCUSSION

How resilient are dune plant communities after invasion for short and long periods?

Acacia longifolia is clearly a transformer (sensu Richardson *et al.* (2000)) converting diverse native areas into species poor, unrecognizable landscapes with several attributes of both biotic and abiotic components altered (Marchante, Marchante & Freitas, 2003; Marchante *et al.*, 2008a, b). This study showed that despite the severe impacts, the removal of almost mono-specific stands of an invader may result in a progressive increase in native species richness, plant cover and life forms revealing inherent resilience within the system. However, as the invasion period extends, the areas become less resilient mainly due to incremental loss of native species. The finding that native species noticeably dominate after clearing, in all situations, suggests that degradation thresholds had not yet been crossed (Aronson *et al.*, 1993; King & Hobbs, 2006) and autogenic recovery occurred to a certain extent with continuous improvement in several ecosystem attributes.

After clearing off the invader, soil microbiology and chemistry (Marchante *et al.*, 2009), light and litter layer conditions, native seed-bank (chapter 4; Marchante, Freitas & Hoffmann, 2010b) and several components of plant communities seemed to slowly recover until they more-closely resembled the preinvasion situation (chapter 2). Although most of the recovering species were generalists, there were also several species characteristic of dunes in the mix, *e.g. Corema album* (L.) Don, *Iberis procumbens* Lange subsp. *procumbens* and *Pseudorlaya minuscule* (Font Quer) Laíz (see Appendix 5.1 for more), species which are relevant for conservation, not only because some are endemic but also because are components of the original communities. Nevertheless, six years after clearing many species characteristic of dunes were still missing, resulting in communities that were not fully representative of the characteristic species assemblages and indicating that dune communities take a long time to return to their initial states, if ever.

Decreased resilience with invasion time occurred similarly in South African 'fynbos' invaded by other *Acacia* species, although species characteristic of the original ecosystem were apparently better represented (Holmes & Cowling, 1997b). Conversely, other post-clearing studies have shown higher abundance of exotic species than natives (Ogden & Rejmánek, 2005; Hulme & Bremner, 2006) and no cumulative increase in species richness with time (Maron & Jefferies, 2001). In fact, the seed-banks of SJDNR had higher abundance of exotic short-lived species (chapter 4; Marchante, Freitas & Hoffmann, 2010b) than is apparent in this field study; this is most likely a mismatch because the few exotics that were abundant as seeds were not able to provide a persistently high cover in the field.

The higher species turnover of recently invaded areas was possibly related to the higher variability of species in the seed banks with probable dependence on different stimuli for germination which was consequently staggered. Soils of recently invaded areas also had lower nutrient content (Marchante *et al.*, 2008b), a condition which is associated with higher species turnover rates (Warren *et al.* 2009).

The recovery process was very dynamic and 6 years after clearing some shifts in species and patterns were obvious, *e.g.,* the area covered by the native shrubs *Cistus salvifolius* L. and *Cytisus grandiflorus* (Brot.) DC. (endemic to Iberian Peninsula) and the invader itself, increased substantially. This was accompanied by a reduction in total plant cover (excluding *A. longifolia*) and diversity due to a substantial decline of some generalist, early coloniser species, mainly *Poaceae* but also *Asteraceae* (Appendix 5.1), which were especially abundant in plots soon after clearing. Some changes observed during recovery would have been missed in a shorter study, corroborating that studies post-clearing of invasive plants should continue for extended periods than most analogous studies (Holmes & Cowling, 1997b; Ogden & Rejmánek, 2005; Hejda & Pyšek, 2006).

Is this system susceptible to (re)invasion after clearing?

Regrowth from cut stumps was negligible in the cleared plots, though clearing in other areas of SJDNR resulted in high levels of resprouting (Marchante, Marchante & Freitas, 2004). The abundance of *A. longifolia* seedlings, particularly in long-invaded cleared plots, made cleared areas susceptible to reinvasion. The densities of seedlings were much lower than the densities of seeds in the soil (chapter 3; Marchante, Freitas & Hoffmann, 2010c) probably due to the absence of fire which stimulates germination (Pieterse & Cairns, 1986).

Most *A. longifolia* seedlings failed to survive to saplings, confirming that estimates of reinvasion potential should be based on established plants instead of seedlings (Galatowitsch & Richardson, 2005). The successful reinvasion by *A. longifolia* was highest on cleared plots where litter (mainly composed of *A. longifolia* abscised phyllodes) remained and consequently retained seeds and elevated soil nutrients (Marchante *et al.*, 2008b). This positive feed-back, where the invader creates conditions that favour itself, has been noted by Milton (1981) and by Ehrenfeld et al (2003).

The cover of *Carpobrotus edulis*, another aggressive invader, also increased after *A. longifolia* removal, particularly in recently invaded areas, thereby representing an additional impediment to native recovery. Being a species with vegetative reproduction (Roiloa *et al.*, 2009), the clearing operations probably fragmented *C. edulis* plants and favoured its spread. Other exotics (*Gamochaeta pensylvanica* (Willd.) Cabrera, *Conyza* spp. and *Cortaderia selloana*) were also found in the cleared areas. Disturbance caused by clearing operations frequently promotes reinvasion by the target species, or by other alien species (Hobbs & Huenneke, 1992; Luken, Kuddes & Tholemeier, 1997; D'Antonio & Meyerson, 2002; Dodson & Fiedler, 2006), emphasizing the need for follow up control (Campbell, 2000; Galatowitsch & Richardson, 2005).

What are the implications of this study for dune ecosystems' management?

In systems where invasions are vast, persistent and include a substantial seed bank of the invader, such as *A. longifolia* in the Portuguese dune ecosystems (Marchante, Marchante & Freitas, 2003; chapter 3; Marchante *et al.*, 2008b, a), eradication is an unrealistic goal (Mack & Lonsdale, 2002). Control measures aimed at reducing the abundance of the invader to less problematic levels and restoring some of the lost structural and functional components of the ecosystem seem feasible, despite hardly being able to recreate the historical landscape. The goal should be to prioritize invaded areas, based on likelihood of successful restoration and on the conservation value of the areas (Wittenberg & Cock, 2005), and then to find the best way to achieve improvement. Our study, as others (Holmes & Cowling, 1997b), recommend that recently invaded areas should be prioritized for control because recovery of both natural vegetation (this study) and soil parameters (Marchante *et al.*, 2009), is likely to be more successful and at less cost. Nonetheless, even in recently invaded areas simply removing the invader does not seems to be sufficient to fully restore the system. Additional manipulation is required (King & Hobbs, 2006), namely planting desirable species, removing the litter and/or depleting the invasive seed bank.

Species that are characteristic of dunes (e.g., *Crucianella maritima* L., *Artemisia campestre* L. subsp. *maritima* Arcangeli, *Helichrysum italicum* (Roth) G. Don fil. subsp. *picardi* (Boiss. & Reuter) Franco) and were recorded as scarce/absent should be sown or transplanted to accelerate recovery, as far as is possible according to the species assemblages typical of each dune community. This has been successfully achieved in some restoration projects (Hartman & McCarthy, 2004). Transplanting will probably be more effective as saplings (cultivated or moved from adjacent dune) will have a height advantage over the invasive seedlings (Galatowitsch & Richardson, 2005), even though this approach might be limited by a lack of available native dune species ready to transplant.

The clearing methods may also have implications for the success of restoration (Holmes & Cowling, 1997b; Holmes *et al.*, 2000). Removing thick litter layers is necessary in systems that are naturally litter poor, such as the dunes (Marchante *et al.*, 2008b). This measure hastened the recovery of native species (Appendix 5.1) and decreased reinvasion potential, particularly in long-invaded areas where there was a more-substantial, slowly-decomposing litter layer (Pereira, Graca & Molles, 1998; Marchante, 2008). Conversely, in recently invaded areas most of the seeds (or other propagules) that germinated soon after clearing were accumulated in the thinner litter layer and were lost with its removal. Lack of seeds in long-invaded litter was probably due to: (i) the disruption of recruitment of new seeds (Holmes & Cowling, 1997b, a); (ii) additional loss of seed viability with time; and (iii) failure of seedlings to establish (Facelli & Pickett, 1991). As the post-clearing period increased, more propagules entered the cleared plots from surrounding areas and the number of species, particularly in recently invaded areas, became increasingly similar in both cleared plots. Litter, even more when the accumulated layer increases, often suppresses germination and establishment of small seeded species (Xiong & Nilsson, 1999; Sayer, 2006) which were frequent in cleared areas. *Myrica faya* and *C. album*, species typical of dunes, and the invader itself, have relatively large seeds whose seedlings survived germination through the deep litter layers. Low-intensity fire could be used to clear *A. longifolia* because it removes the nitrogen-rich litter and scorches seeds thereby depleting the seed bank (Holmes & Cowling, 1997b).

The size of the area that is cleared apparently influences the recovery process, with resurgence of *A. longifolia* less likely and successful recovery more probable when small areas are cleared. Subsequently cleared areas may be progressively increased. Under natural conditions smaller gaps disappear more rapidly than larger gaps through encroachment of the surrounding vegetation (Fenner & Thompson, 2005). Additionally, small scale clearing operations generate lower levels of disturbance being less of a stimulus to germination of *A. longifolia* seeds. Clearing operations over several hectares that took place elsewhere in SJDNR resulted in a much higher (sometimes over 70% *A. longifolia* cover) and more rapid (less than two years) reinvasion.

In conclusion, sand dunes densely invaded by woody species have inherent resilience which decreases as invasion time extends. Regardless of time of invasion, autogenic recovery of cleared areas can be enhanced by active management actions which maximise, and generate synergy between abiotic and biotic components of the system (King & Hobbs, 2006). For example, the initial clearing of thickets of the invasive species and the additional removal of the litter layer (practice that may be improved if achieved with fire that additionally reduces the invader seed bank) foster abiotic changes, *e.g.,* light increase, decrease of barriers to germination and disruption of N addition. Such changes will favour desirable, and a few undesirable, species. Subsequent transplantation of missing native species can be used to further encourage re-establishment of the natural communities and simultaneously reduce subsequent reinvasion (Hulme, 2006). However, encroachment of the invader after initial control will be vigorous and needs to be further curtailed. This can be done on a sustainable basis by introducing a biological control agent, as *Trichilogaster acaciaelongifoliae*, which has been successfully used for that purpose in South Africa and is being studied in Portugal (Dennill *et al.*, 1999; Marchante, Freitas & Hoffmann, 2010a; chapter 6). The full recovery of ecosystems to their original state (both structurally and functionally) may be very long-lasting and thus an unrealistic restoration target.

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Appendix 5. 1

Plant species detected in invaded areas (long- and recently invaded) monitored in plots subject to clearing treatments (AR – *A. longifolia* removed or ALR – *A. longifolia* + litter both removed). Numbers in the table correspond to: 1) no. of observations of the species per plot (ob. - from 2002 to 2008), and mean plot cover in the last sampling (May 2008).

			Recently-Invaded areas						**Long-Invaded areas**					
N/			A		AR		ALR		A		AR		ALR	
/E	**Species**	**Treatments:**	ob.	2008	ob.	2008	ob.	2008	ob.	2008	ob.	2008	ob.	2008
Species detected exclusively in recently-invaded areas (27):														
N	*Pseudorlaya minuscula* (Font Quer) Laínz *	*Apiaceae*	- -	- -	- -	- -	2	- -						
N	*Chamaemelum mixtum* (L.) All.	*Asteraceae*	- -	- -	5	- -	9	0.14						
N	*Coleostephus myconis* (L.) Reichenb. fil.	*Asteraceae*	- -	- -	- -	- -	2	- -						
N	*Hirschfeldia incana* (L.) Lagrèze-Fossat	*Brassicaceae*	- -	- -	3	- -	- -	- -						
N	*Iberis procumbens* Lange *procumbens* *	*Brassicaceae*	- -	- -	- -	- -	1	- -						
N	*Malcolmia ramosissima* (Desf.) Thell. *	*Brassicaceae*	- -	- -	- -	- -	12	0.18						
N	*Teesdalia nudicaulis* (L.) R. Br.	*Brassicaceae*	2	- -	21	0.69	18	0.88						
N	*Cerastium glomeratum* Thuill.	*Caryophyllaceae*	- -	- -	5	- -	6	- -						
N	*Corrigiola litoralis* L.	*Caryophyllaceae*	- -	- -	- -	- -	1	- -						
N	*Sagina apetala* Ard. *erecta* (Hornem.) F. Hermann	*Caryophyllaceae*	- -	- -	4	- -	14	0.03						
N	*Scleranthus annuus* L.	*Caryophyllaceae*	- -	- -	- -	- -	5	- -						
N	*Silene gallica* L.	*Caryophyllaceae*	- -	- -	8	0.12	15	0.60						
N	*Spergula arvensis* L.	*Caryophyllaceae*	- -	- -	2	- -	5	- -						
N	*Stellaria media* (L.) Vill.	*Caryophyllaceae*	- -	- -	1	- -	1	- -						
N	*Cytisus grandiflorus* (Brot.) DC.	*Fabaceae*	1	- -	11	0.45	13	4.39						
N	*Ornithopus pinnatus* (Miller) Druce	*Fabaceae*	- -	- -	5	- -	6	- -						
N	*Vicia sativa* L.	*Fabaceae*	- -	- -	- -	- -	1	- -						
N	*Geranium molle* L.	*Geraniaceae*	- -	- -	- -	- -	2	- -						
N	*Juncus capitatus* Weigel	*Juncaceae*	- -	- -	- -	- -	4	0.04						
N	*Stachys arvensis* (L.) L.	*Lamiaceae*	- -	- -	- -	- -	4	- -						
N	*Epilobium tetragonum* L.	*Onagraceae*	- -	- -	3	- -	2	- -						
E	*Oxalis pes-caprae* L.	*Oxalidaceae*	- -	- -	4	- -	4	- -						
N	*Catapodium marinum* (L.) C.E. Hubbard *	*Poaceae*	- -	- -	3	- -	4	0.03						
N	*Anagallis arvensis* L.	*Primulaceae*	- -	- -	4	0.09	7	0.12						
N	*Asterolinum linum-stellatum* (L.) Duby	*Primulaceae*	- -	- -	6	0.08	8	0.02						
N	*Lonicera periclymenum* L.	*Caprifoliaceae*	**1**	0.02	- -	- -	- -	- -						
N	*Trifolium arvense* L. var. *arvense*	*Fabaceae*	- -	- -	- -	- -	2	0.03						
Species detected exclusively in long-invaded areas (6):														
N	*Juniperus phoenicea* L	*Cupressaceae*							1	- -	- -	- -	- -	- -
N	*Corema album* (L.) D. Don *	*Empetraceae*							3	- -	8	- -	14	0.75
N	*Quercus robur* L.	*Fagaceae*							**- -**	**- -**	**1**	**- -**	**- -**	**- -**
N	*Juncus acutus* L.	*Juncaceae*							- -	- -	1	- -	- -	- -
N	*Polypodium vulgare* L.	*Polypodiaceae*							- -	- -	14	0.25	- -	- -
N	*Antirrhinum majus* L. *cirrhigerum* (Ficalho) Franco *	*Scrophulariaceae*							- -	- -	1	- -	4	0.12

			Recently-Invaded areas						Long-Invaded areas					
N/			A		AR		ALR		A		AR		ALR	
/E	**Species**	**Treatments:**	ob.	2008	ob.	2008	ob.	2008	ob.	2008	ob.	2008	ob.	2008
Species detected in long- and recently-invaded areas (50):														
E	*Carpobrotus edulis* (L.) N. E. Br.	*Aizoaceae*	12	- -	20	3.20	30	13.11	- -	- -	4	0.20	31	2.37
N	*Andryala integrifolia* L.	*Asteraceae*	9	- -	52	2.78	55	3.71	- -	- -	31	1.47	47	1.06
E	*Conyza* sp.	*Asteraceae*	9	0.03	27	0.42	40	0.87	2	- -	22	0.08	24	0.04
N	*Crepis capillaris* (L.) Wallr.	*Asteraceae*	2	0.01	9	5.15	8	1.05			3	1.47		
E	*Gamochaeta pensylvanica* (Willd.) Cabrera	*Asteraceae*	1	0.02	4	- -	11	- -	- -	- -	- -	- -	1	- -
N	***Hypochaeris glabra* L.**	*Asteraceae*	- -	- -	37	0.96	53	1.50	1	0.02	29	2.12	41	2.71
N	*Lactuca virosa* L.	*Asteraceae*	- -	- -	9	- -	3	- -	2	0.04	6	0.06	9	0.12
N	*Logfia minima* (Sm.) Dumort.	*Asteraceae*	- -	- -	23	0.48	44	0.71	- -	- -	2	- -	33	0.42
N	*Picris echioides* L.	*Asteraceae*	2	- -	3	- -	5	- -	- -	- -	5	- -	12	- -
N	*Pseudognaphalium luteo-album* (L.) Hilliard & B.L.Burtt	*Asteraceae*	- -	- -	4	- -	1	- -	- -	- -	- -	- -	2	- -
N	*Senecio lividus* L.	*Asteraceae*	3	0.26	9	0.35	4	0.05	10	0.06	6	0.08	12	0.44
N	*Senecio vulgaris* L.	*Asteraceae*	21	0.12	48	0.55	60	0.88	11	0.08	35	0.16	49	0.48
N	*Sonchus asper* (L.) Hill	*Asteraceae*	2	- -	1	- -	1	- -	- -	- -	- -	- -	1	- -
N	*Sonchus oleraceus* L.	*Asteraceae*	34	0.10	57	0.47	34	- -	7	0.16	31	0.28	42	0.34
N	*Sonchus tenerrimus* L.	*Asteraceae*	1	0.04	5	0.33	7	0.48	- -	- -	- -	- -	4	0.08
N	*Urospermum picroides* (L.) F. W. Schmidt.	*Asteraceae*	8	- -	- -	- -	4	0.12	- -	- -	2	- -	2	- -
N	*Cardamine hirsuta* L.	*Brassicaceae*	9	- -	21	- -	15	0.03	14	- -	16	0.04	29	0.10
N	*Cerastium diffusum* Pers. *diffusum*	*Caryophyllaceae*	1	- -	6	- -	21	0.06	- -	- -	2	0.32	10	0.70
N	*Polycarpon tetraphyllum* (L.) L.	*Caryophyllaceae*	6	0.04	25	1.02	29	0.63	- -	- -	- -	- -	3	- -
N	*Silene longicaulis* Lag.	*Caryophyllaceae*	- -	- -	7	0.83	14	3.83	- -	- -	- -	- -	10	0.46
N	*Silene micropetala* Lag. *	*Caryophyllaceae*	- -	- -	7	0.12	14	- -	- -	- -	- -	- -	2	0.02
N	*Cistus psilosepalus* Sweet	*Cistaceae*	- -	- -	1	- -	- -	- -	- -	- -	7	0.58	2	- -
N	*Cistus salvifolius* L.	*Cistaceae*	21	0.08	51	11.49	36	8.17	23	0.22	41	8.38	62	37.55
N	*Tuberaria guttata* (L.) Fourr.	*Cistaceae*	- -	- -	22	1.52	19	1.03	- -	- -	7	0.06	20	0.57
N	***Carex arenaria* L.***	*Cyperaceae*	- -	- -	- -	- -	1	- -	2	0.45	14	3.69	13	0.75
E	*Acacia longifolia* (Andrews) Willd.	*Fabaceae*	70	66.47	51	17.38	50	8.85	70	87.15	55	18.54	60	12.71
N	*Ulex europaeus* L. *latebracteatus* (Mariz) Rothm. *	*Fabaceae*	15	2.21	13	0.38	6	- -	28	1.96	30	2.86	36	1.84
N	*Geranium purpureum* Vill.	*Geraniaceae*	5	- -	24	0.56	18	0.27	2	0.06	8	0.44	- -	- -
N	*Myrica faya* Aiton *	*Myricaceae*	- -	- -	1	0.02	4	0.08	6	0.10	20	3.47	1	- -
N	*Fumaria muralis* Koch subsp. *muralis*	*Papaveraceae*	2	0.01	13	0.34	8	0.12	12	0.52	3	0.06	11	0.19
N	*Pinus pinaster* Aiton	*Pinaceae*	1	0.04	17	2.76	15	0.38	19	0.81	31	5.60	23	1.70
N	*Agrostis stolonifera* auct. lusit., non L. *pseudopungens* (Lange) Kerguélen *	*Poaceae*	- -	- -	16	0.64	26	0.98	- -	- -	17	0.86	23	0.80
N	***Aira praecox* L.**	*Poaceae*	9	0.19	39	0.95	43	1.13	- -	- -	18	1.93	39	2.70
N	*Avena barbata* Link *barbata*	*Poaceae*	- -	- -	- -	- -	2	- -	- -	- -	1	0.06	- -	- -
N	***Briza maxima* L.**	*Poaceae*	24	3.37	35	1.80	21	2.08	10	0.16	26	14.96	29	1.34
N	*Briza minor* L.	*Poaceae*	- -	- -	2	- -	3	- -	- -	- -	1	- -	- -	- -
N	*Bromus diandrus* Roth	*Poaceae*	- -	- -	4	0.09	- -	- -	- -	- -	5	- -	2	- -
N	***Bromus rigidus* Roth**	*Poaceae*	4	- -	55	1.33	49	0.95	11	- -	17	4.14	22	1.39
E	*Cortaderia selloana* (Schult. & Schult.F) Asch. & Graebn	*Poaceae*	3	- -	2	- -	- -	- -	1					
N	*Corynephorus canescens* (L.) Beauv.	*Poaceae*	- -	- -	2	- -	5	- -	- -	- -	- -	- -	1	- -
N	*Dactylis glomerata* L. *hispanica* (Roth) Nyman	*Poaceae*	- -	- -	6	0.37	2	- -	27	0.25	12	1.39	5	0.08
N	*Lagurus ovatus* L. *	*Poaceae*	1	- -	3	0.09	1	- -	- -	- -	- -	- -	4	0.10
N	***Lolium* sp.**	*Poaceae*	- -	- -	- -	- -	- -	- -	- -	- -	7	2.19	8	0.58
N	*Vulpia alopecuros* (Schousboe) Dumort. *alopecuros* *	*Poaceae*	- -	- -	19	1.52	12	1.11	- -	- -	8	0.64	9	0.80
N	***Vulpia bromoides* (L.) S.F. Gray**	*Poaceae*	30	3.62	66	2.47	63	4.97	8	0.10	22	0.53	42	3.18
N	***Vulpia membranacea* (L.) Dumort.**	*Poaceae*	- -	- -	15	1.01	15	1.99	- -	- -	8	0.40	18	1.23
N	*Rumex bucephalophorus* L.	*Polygonaceae*	- -	- -	- -	- -	1	- -	- -	- -	1	0.06	7	0.33
N	*Galium minutulum* Jordan	*Rubiaceae*	- -	- -	2	0.08	3	0.14	9	- -	4	- -	- -	- -
N	*Solanum nigrum* L. *nigrum*	*Solanaceae*	2	0.01	1	- -	6	- -	- -	- -	6	- -	8	- -
N	*Centranthus calcitrapa* (L.) Dufresne *calcitrapa*	*Valerianaceae*	4	0.03	7	0.03	2	- -	- -	- -	5	- -	4	- -
N	*Sonchus* sp.	*Asteraceae*	5	0.08	4	- -	- -	- -	- -	- -	7	0.76	- -	- -
	Poaceae 1	*Poaceae*	4	- -	- -	- -	- -	- -	1	- -	- -	- -	- -	- -
	Poaceae 2	*Poaceae*	2	- -	4	- -	4	- -	1	- -	- -	- -	3	- -
	Asteraceae	*Asteraceae*	- -	- -	3	- -	2	- -	4	- -	1	- -	5	- -
	Magnoliopsida (seedlings)		1	- -	4	- -	4	- -	- -	- -	3	- -	5	- -
number of species per treatment/invasion age:			**36**	**22**	**64**	**40**	**74**	**42**	**26**	**18**	**48**	**36**	**49**	**36**
number of species per invasion age:								**81**						**61**

N = native; E = exotic; ***** species characteristic from dune ecosystems; species **at bold** had their cover markedly decreased by May 08
(species that were ephemeraly observed where registere in ob. but no longer present in the last monitoring (May 2008).

Chapter 6

Assessing the suitability and safety of a well-known bud-galling wasp, *Trichilogaster acaciaelongifoliae*, for biological control of *Acacia longifolia* in Portugal

ABSTRACT

Acacia longifolia is a widespread invasive plant species in Portugal. In South Africa, its spread was controlled by a bud-galling wasp, *Trichilogaster acaciaelongifoliae*, a strategy that could also be applied in Portugal. Biological control of invasive alien plants has received little consideration anywhere in Europe and has never been attempted in Portugal. The lack of a suitably-large quarantine facility necessitated the use of a novel approach to test non-target species in Portugal. With authorization from the Institute for Conservation of Nature and Biodiversity (ICNB), mature *T. acaciaelongifoliae* galls were shipped to Portugal from South Africa to obtain adult female wasps, which were then confined in Petri dishes each with a bud-bearing branch of one of 40 non-target plant species. The time spent by the wasps exploring and probing the buds was measured and after that buds were dissected to detect any egg deposition. Species where eggs were found were then submitted to additional tests. The results showed that *T. acaciaelongifoliae* did not respond to the buds of most (23) species. The females spent time on the buds of the other 17 species but only laid eggs in three species besides *A. longifolia.* Oviposition on *A. melanoxylon* was expected but it was not anticipated on vines, *Vitis vinifera*, (where eggs were deposited externally in the pubescent coat of the buds) or on broom, *Cytisus striatus*, (where eggs were inserted into the buds the same way they are on *A. longifolia*). Paired-choice with these three non-target species showed that no eggs were laid on *V. vinifera* and that less than two eggs/branch, on average, were laid on *C. striatus* and *A. melanoxylon*. Subsequent trials on potted plants showed that galls only developed on *A. longifolia.* Field surveys in South Africa and Australia showed that galls never occur on either vines or broom. The implications of these findings for biological control of *A. longifolia* in Portugal with *T. acaciaelongifoliae* are considered in relation to the wealth of experience and knowledge about the specificity of the wasp and the reliability of conducting host-specificity tests under confined conditions of cages. A brief overview of the legal procedures needed to secure the release of *T. acaciaelongifoliae* in Portugal is also presented.

Key words: *Acacia longifolia,* biocontrol, buds dissection, Europe, invasive plant species, specificity tests, Sydney golden wattle, *Trichilogaster acaciaelongifoliae*.

INTRODUCTION

Acacia longifolia (Andrews) Willd. (Sydney golden wattle, long-leaved wattle) is a small leguminous tree or shrub, native to south-eastern Australia, which is invasive both in Portugal (Marchante, 2001;

Marchante, Marchante & Freitas, 2003) and South Africa (Dennill *et al.*, 1999), as well as other regions of the globe (Elorza, Sánchez & Vesperinas, 2004). Some authors (Whibley, 1980) recognize two subspecies within this taxon: *A. longifolia* (Andrews) Willd. subsp. *longifolia* and *A. longifolia* (Andrews) Willd. subsp. *sophorae* (Labill.) Court., while others treat *A. longifolia* and *A. sophorae* (Labill.) R. Br. as distinct species (Paiva, 1999). *Acacia floribunda* (Vent.) Willd is closely related to *A. longifolia,* being considered in the past as a subspecies of *A. longifolia* (Maslin, 2001).

Acacia longifolia was first introduced to Portugal in the late 19th century to curb sand erosion along coastal dunes (Neto, 1993). Since then *A. longifolia* has spread into other areas, both naturally and by horticulturists who favor its bright yellow flowers (Almeida, 1999; Kull *et al.*, submitted). It grows rapidly and has prolific production of seeds which accumulate in the soil, reaching average levels of 1500 seeds.m^{-2} (chapter 3; Marchante, Freitas & Hoffmann, 2010b).The seeds respond to fire and germinate *en masse* in the ash beds (Pieterse & Cairns, 1988). With time, extensive thickets have formed in coastal sand dunes and a variety of other habitats, particularly along rivers, road edges and on mountain slopes (Marchante, Freitas & Marchante, 2008). *Acacia longifolia* is legally considered as an invasive species in Portugal (Ministério do Ambiente, 1999). Its ability to fix nitrogen (Rodríguez-Echeverría, Crisóstomo & Freitas, 2007), and the absence of natural enemies, contribute to making *A. longifolia* a highly competitive species capable of shading out native species (Marchante, Marchante & Freitas, 2003) and posing a substantial threat to local biodiversity (Marchante, 2001) while changing soil properties and altering ecosystem processes (chapter 3; Marchante *et al.*, 2008b, a, 2009).

In Portugal, control of *A. longifolia* relies on mechanical methods, mainly basal cutting and, to a less extent, on chemical application of herbicide to the cut ends of the stumps. These methods are prohibitively expensive and have failed to achieve lasting control (Marchante, Marchante & Freitas, 2004), mostly due to replenishment of thickets from the abundant seed banks in the soil (Marchante, Freitas & Hoffmann, 2010a).

In South Africa, where *A. longifolia* had been problematic for over a hundred years, biological control with an Australian gall wasp, *Trichilogaster acaciaelongifoliae* Froggatt (Hymenoptera: Pteromalidae), later assisted by a seed-feeding weevil *Melanterius ventralis* Lea (Coleoptera: Curculionidae), has proven to be an excellent management option (Dennill, 1988*;* Impson & Moran, 2003) which is not yet available in Portugal.

The first *T. acaciaelongifoliae* individuals introduced to South Africa, during 1982, were collected in Australia from its two known, closely-related hosts, *A. longifolia* and *A. floribunda* (Dennill, 1987).

Soon after release, it became clear that besides having a direct effect on seed production (Dennill, 1990; Dennill *et al.*, 1999), *T. acaciaelongifoliae* galls also act as nutrient sinks and thereby indirectly inhibit the development of both reproductive and vegetative growth of their host plants, often causing die back of branches and whole plants when environmental conditions are harsh (Dennill, 1985; Impson & Moran, 2003). There have been no quantitative surveys to demonstrate the overall effectiveness of the wasp as a biocontrol agent (Hoffmann *et al.*, 2002) but *A. longifolia* is generally no longer considered to be anywhere near as problematic as it was formerly, a change that is attributed to biological control having succeeded in South Africa.

Recently, consideration has been given to using *T. acaciaelongifoliae* in Portugal for biological control of *A. longifolia.* The prospects of success are good because the wasp has a proven track record and because it is highly host specific. The specificity of *T. acaciaelongifoliae* was confirmed before it was released in South Africa by exposing potted plants to the insects under caged quarantine conditions and subsequently seeking signs of gall development in non-target plants (Van den Berg, 1980; Dennill, Donnelly & Chown, 1993). Since its release, *T. acaciaelongifoliae* only utilizes two of its known Australian host plants, *A. longifolia* and *A. floribunda* (Dennill & Donnelly, 1991; McGeoch & Wossler, 2000). Underdeveloped galls are very rarely seen on *Paraserianthes lophantha* (Willd.) Nielsen and *Acacia* melanoxylon R. Br. in South Africa, but only when these plants occur in close proximity to heavily galled *A. longifolia* plants and neither of the two species is considered to be a suitable host for the wasp (Dennill, Donnelly & Chown, 1993). The host specificity of *T. acaciaelongifoliae* is not unexpected because insects that attack and live within the reproductive parts of their host plant (especially gall-forming insects) almost always display a high degree of monophagy (Ananthakrishnan, 1984).

Although classical biological control has been used against insect pests in Europe (EPPO, 2008), to date only one biological control agent (the psyllid, *Aphalara itadori* Shinji) has been approved to release against an alien invasive plant (*Fallopia japonica* (Houtt) R. Decr.), which occurred in 2010 (Sheppard, Shaw & Sforza, 2006; Shaw, Bryner & Tanner, 2009; EPPO, 2010). Despite the unequivocal body of evidence that *T. acaciaelongifoliae* is highly host specific (Dennill, Donnelly & Chown, 1993), due to the novelty of the process in Europe, regulatory authorities in Portugal insisted that additional evidence should be obtained to confirm that *T. acaciaelongifoliae* will not inflict any damage on non-target hosts, noting that the insects would encounter a distinctive suite of plants in a different hemisphere.

The lack of a suitably-large quarantine facility to perform host specificity tests in potted plants presented a challenge in determining how the wasps would respond to the plant species on the list that was drawn up. This chapter describes the methods that were used, the results that were obtained and the implications of the findings for deciding whether or not *T. acaciaelongifoliae* should be cleared for release in Portugal. An overview of the legal procedures needed to secure the release of *T. acaciaelongifoliae* in Portugal is also presented.

MATERIALS AND METHODS

Biology of agent and host plant - The biology of *T. acaciaelongifoliae* has been described by Noble (1940) and Dennill (1985; 1987). It is a small (ca. 3mm in length), parthenogenic, univoltine bud-galling wasp that deposits its eggs within the tissues of both reproductive and vegetative buds of *A. longifolia* thereby inducing galls which prevent flowering and curb branch growth (Dennill, 1985). In the southern hemisphere (including both the native range in Australia and the exotic range in South Africa), *T. acaciaelongifoliae* adults emerge predominantly in spring and early summer (October to December/January) and immediately commence oviposition (Dennill, 1987) (Figure 6.1). A small proportion of adults emerge at other times of the year (N. Dorchin, *personal communication*). The females live for only 2 - 3 days, do not feed and spend most of their time searching for suitable buds on which to lay their eggs. Adult females are highly fecund (409 eggs/female in average) and disperse readily.

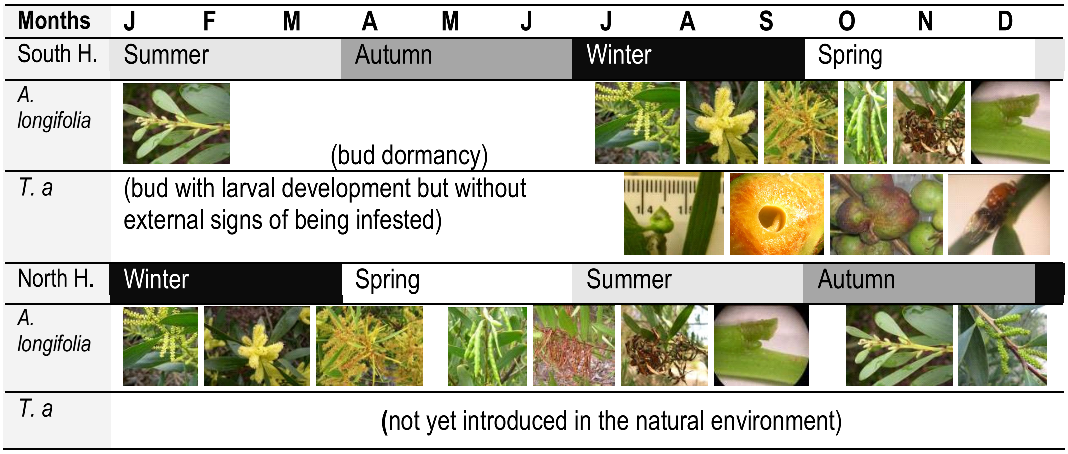

Figure 6.1 The annual life cycle of *T. acaciaelongifoliae* (T. a) in relation to the reproductive phenology of *A. longifolia*, in Southern (South H.) and Northern (North H.) Hemisphere. Pictures illustrate the average time of occurrence of each phenophase although delays and advances are usually present both within and amongst populations of different localities.

The galls develop during spring (late July to beginning of October, in South Hemisphere, when *A. longifolia* flowers), in the axils of the phyllodes of *A. longifolia.* At this stage they are mostly green and spherical (1-4mm in diameter) (Figure 6.1), being single (one chamber) or in clusters (multichambered). As the galls mature they increase in size and by December they are fully developed reddish green in colour. Once the wasps emerge galls desiccate, shrivel and turn brown. Galls typically contain between 1 and 7 chambers (with up to 24 chambers possible), depending on the number of eggs originally laid in the bud. The eggs lie dormant until late winter when they hatch and multilocular galls start to develop. Each larva has a discrete chamber in which it completes its development. Most chambers contain females but occasional males develop in smaller chambers on the periphery of the gall. The sex ratio of females per male averages 5.3:1 (Dennill, 1987).

Milton and Moll (1982) studied the phenology of *A. longifolia* in South Africa and showed that while the timing of events varies with habitat and location, there is active vegetative growth on the plants from September to December (spring and early summer) and then again in autumn (April-May). The flower buds are set as the new growth forms but flowers only develop between August and November after a period of bud dormancy in winter. Pods develop from September to November and are fully ripe by mid-November. The period when young buds are suitable for *T. acaciaelongifoliae* oviposition overlaps with the period when galls are maturing and pods are ripening. In Portugal, the phenology of *A. longifolia* also varies in the different regions where the plants occur and shows some seasonal differences to the southern hemisphere (M. Morais, *unpublished data*). Usually, first flowers are observed in December but full bloom is in February - March (late winter through to the beginning of spring). Pods develop from March to July and ripen between June and August. Small buds (1-3 mm) dominate in June-August but are still present in lower numbers until December. Vegetative growth occurs predominantly from April to August (spring and summer) (Morais & Freitas, 2008). Trees in more northern regions show a slightly delayed cycle.

Specificity test plant list - The plant species to be included in non-choice tests were selected according to criteria outlined by Briese (2002) and Briese and Walker (2002), including phylogenetic proximity and morphological similarity (specifically bud structure) to *A. longifolia*. Other factors considered were: economic value, conservation importance (*e.g.* endemic species), and biogeographic and ecological overlap (*i.e.* plants that are common in sand dunes, the habitat most frequently invaded by *A. longifolia*). The selection included 40 species (Table 6.1) that fulfilled either one (*e.g. Quercus faginea* Lam.) or several (*e.g. Stauracanthus genistoides* (Brot.) Samp.) of the selection criteria. The final plant list was approved independently by ICNB (Portuguese Institute for Nature & Biodiversity Conservation), who had nominated some of the species on the list. The degree of phylogenetic separation between the listed plants and *A. longifolia* was established following Judd *et al.* (1999), mainly to determine higher level of phylogeny (families, orders and major clades).

Table 6.1 List of plant species tested in non-choice tests with *Trichilogaster acaciaelongifoliae*, including selection criteria for each species. (n = native species; e = exotic species).

Family			**Non-target species**	**criteria**
Anacardiaceae	1	n	*Pistacia lentiscus* L.	□ ▒
Caprifoliaceae	2	n	*Viburnum tinnus* L.	□ ▒ ■
Cistaceae	3	n	*Cistus psilosepalus* Sweet	□ ■
Empetraceae	4	n	*Corema album* (L.) D.Don	□ ■
Ericaceae	5	n	*Arbutus unedo* L.	□ ▒ ■
	6	n	*Erica scoparia* L.	□ ■
Fabaceae	7	e	subfam. *Caesalpinioideae - Ceratonia siliqua* L.	▨ ▩
(=Leguminosae)	8	n	subfam. *Faboideae - Cytisus striatus* (Hill.) Rothm.	▨ □ ▒ ■
	9	n	subfam. *Faboideae - Genista falcata* Brot.	▨ □ ▒
	10	n	subfam. *Faboideae – Medicago marina* L.	▨ □ ■
	11	e	subfam. *Faboideae - Phaseolus vulgaris* L.	▨ ▩
	12	e	subfam. *Faboideae - Pisum sativum* L.	▨ ▩
	13	n	*subfam. Faboideae - Stauracanthus genistoides* (Brot.) Samp. subsp. g*enistoides*	▨ □ ▒ ■
	14	n	subfam. *Faboideae - Ulex parviflorus* L.	▨ □ ■
	15	e	subfam. *Faboideae - Vicia faba* L.	▨ ▩
	16	e	subfam. *Mimosoideae - Acacia melanoxylon* R. Br.	▨ ▒ ■
Fagaceae	17	n	*Quercus faginea* Lam.	□
	18	n	*Quercus lusitanica* Lam.	□
	19	n	*Quercus pyrenaica* Willd.	□
	20	n	*Quercus robur* L.	□ ▩ ■
	21	n	*Quercus rotundifolia* Lam.	□ ▩
	22	n	*Quercus suber* L.	□ ▩ ■
	23	n	*Quercus x coutinhoi* Samp.	□
Lamiaceae	24	n	*Lavandula luisieri* (Rozeira) Rivas-Martinez	□ ■
Lauraceae	25	n	*Laurus nobilis* L.	□ ▒ ■
Myricaceae	26	n*	*Myrica faya* Aiton	□ ▒ ■
Myrtaceae	27	e	*Eucalyptus globulus* Labill.	▩
Oleaceae	28	n	*Phillyrea angustifolia* L.	□ ▒ ■
Pinaceae	29	n*	*Pinus pinaster* Aiton	▩ □ ■
	30	e	*Pseudotsuga menziesii* (Mirbel) Franco	▩
Polygalaceae	31	n	*Polygala vulgaris* L.	▨ □
Rhamnaceae	32	n	*Rhamnus alaternus* L.	□ ▒ ■
Rosaceae	33	e	*Pyrus communis* L.	▩
	34	e	*Prunus persica* (L.) Batsch.	▩
	35	n	*Prunus lusitanica* L.	□ ▒
	36	e	*Malus domestica* Borkh.	▩
Rutaceae	37	e	*Citrus sinensis* (L) Osbeck	▩ ▒
Salicaceae	38	n	*Salix atrocinerea* Brot.	□ ■
Ulmaceae	39	n	*Ulmus procera* Salisb.	□ ■
Vitaceae	40	e	*Vitis vinifera* L.	▩

species phylogentically related (centrifugal phylogenetic method) ▨
species with some morphological (buds, *i.e.* size, absence of indument,...) similarities ▒
species with ecological/distribution overlap ■
economic plant species ▩
species with conservation value □

* Considered to be exotic by some authors

Note: Where more than one criteria was used for selection of a particular species, the relative importance of each of the criteria is indicated by the width of the blocks

Congeneric species were not included in the test list, with the exception of *A. melanoxylon*, because: a) there are no congeneric native species (or any other *Mimosoideae*) in Portugal or elsewhere in Western Europe; b) none of the introduced *Acacia* spp. has major economic value in Portugal; and c) several *Acacia* species (*A. baileyana* F. Muell.; *A. cyclops* A.Cunn. ex G.Don; *A. dealbata* Link; *A. decurrens* (J.C. Wendl.) Willd.; *A. floribunda* (Vent.) Willd; *A. mearnsii* De Wild; *A. melanoxylon* and *A. saligna* (Labill.) H.L. Wendl.) were subjected to host specificity tests in South Africa where galls only developed on *A. floribunda*, a recognized host plant of *T. acaciaelongifoliae* in its native range, besides *A. longifolia. Acacia melanoxylon* was included in the tests to confirm the status of infrequent observations of sporadic gall formation on this plant species in South Africa.

The test species were separated into six categories on the basis of their phenology. The groups comprised the target invader *A. longifolia,* and five clades with increasing phylogenetic distance from the target (see Figure 6.3), including: 1) species from the genus *Acacia*; 2) species from other genera within the family *Fabaceae*; 3) species from other families within the Order *Fabales* namely *Polygalaceae*; 4) species from more distant related families within the *Rosidae* (specifically clade Eurosids I, which includes the *Fabaceae*), namely *Rosaceae, Salicaceae, Rhamnaceae, Ulmaceae, Fagaceae* and *Myricaceae*; and 5) species from distant families outside the Eurosids I. Although some authors (Heywood, 1993; Izco *et al.*, 1998) consider the Order *Fabales* to be monophyletic, including *Fabaceae* alone, others (Judd *et al.*, 1999) recognize three families in the order, based on morphological characters and rbcL sequences, with the *Polygalaceae* being the only family with species present in Portugal.

Three annual species (*Vicia faba* L., *Pisum sativum* L. and *Phaseolus vulgaris* L.) were included on the list even though the wasp needs an entire year to complete its development within its gall, a mismatch which will preclude this group of plants as possible hosts. The three species where included because they belong to the same family as *A. longifolia* and due to their importance as crops.

Host specificity testing - *Trichilogaster acaciaelongifoliae* galls were collected from late September to December during 2005, 2006, 2007, 2008 and 2010 on the campus of the University of Cape Town, South Africa (33º57'S 18º27'E). For shipment, batches of galls were packaged in sealed polyester cloth bags inside cardboard containers which were air freighted to Portugal. The packaging allowed exchange of respiratory gases while ensuring containment of any insects that emerged in transit. The galls were received at Escola Superior Agrária de Coimbra (Portugal), where they were kept in a quarantine facility at approximately 25ºC, 12: 12 L: D, conditions which were maintained before and during experiments. *Acacia longifolia* was collected from several localities in Coimbra

(40º20'N, 8º40'W) and S. Jacinto Dunes (40º39'N, 8º44'W). Branches of test plants were collected in several locations immediately before initiation of the test and were transported with the cut end of the stem in a container of water to prevent wilting. In the laboratory, shoots with small buds had the cut end covered with damp tissue paper which was held in place with aluminum foil.

No-choice tests - The behavior and relative acceptability of all non-target plant species as oviposition sites for *T. acaciaelongifoliae* was assessed in no-choice tests with *A. longifolia* as a control. Each shoot was placed in a Petri dish (5 cm tall, 23 cm diameter) and exposed to one female wasp for the duration of its adult life (2 to 3 days). Nine branches were tested per species, each with a separate wasp in an individual cage. Whenever possible, each cage contained plants with comparable amounts of foliage and numbers of buds (frequently, seven or more buds). Some exceptions were inevitable due to distinct plant morphology, namely species with high numbers of small buds in close proximity to each other along the shoot (e.g., *Erica scoparia* L. and *Corema album* (L.) D. Don) or species with buds widely spaced along the shoot (e.g., *Ceratonia siliqua* L. and *Pinus pinaster* Aiton).

Paired-choice tests were conducted in November 2010 to evaluate preference by *T. acaciaelongifoliae* females on *A. longifolia* paired with each of the non-target species where eggs were detected in non-choice tests. Tests were conducted in the same Petri dishes as non-choice tests, for the duration of the females' adult life. Nine pairs of branches, of equivalent size, were tested per non-target species.

Observations of the behavior of T. acaciaelongifoliae - Behavior of each wasp was observed during nine observation sessions of 1200 s (20 minutes) for each test (including non-choice and paired-choice). An observation session commenced when the wasp first moved on to a plant or after five minutes if this had not happened by then. Over the five years of the trials there were 131 h of observations of the wasps.

Dissection of buds to detect T. acaciaelongifoliae eggs - After exposure to the female wasps, buds of branches used in no-choice and paired-choice tests were characterized according to size (< 1 mm; 1 mm; 1.5 mm; 2 mm; ≥ 3 mm). In all the branches, from both no-choice and paired-choice tests, buds were dissected under a binocular microscope to determine the number of *T. acaciaelongifoliae* eggs that had been deposited, if any. At least seven buds (exceptionally less in species with fewer buds per mm of shoot) were dissected per branch. Eggs of *T. acaciaelongifoliae* are minuscule (approximately 0.2 mm in length), brilliant white and recognizable by their oval to oblanceolate shape (Figure 6.2).

Figure 6.2 *Acacia longifolia* (left) and *Vitis vinifera* (right) buds dissected to reveal several *T. acaciaelongifoliae* eggs. On *A. longifolia* buds the eggs were enclosed in bud tissue. On *V. vinifera* the eggs were lodged in the protective layer of pubescence that covers the surface of the bud.

Gall induction on potted plants - Plant species in which eggs were detected in buds were subsequently tested further, except for *A. melanoxylon*. This species was not included because it was particularly difficult to get the small potted plants needed for the experiment and it is already known to support gall formation sporadically, *i.e.,* observations in the field in South Africa confirmed the result of the oviposition test. For each species, six small (30-90 cm) potted plants were enclosed separately in a plastic bag into which two adult *T. acaciaelongifoliae* females were added and left until they died, corresponding to ca. two days of contact. Wasps were transferred to the potted plant within 14 h of emergence. Two days after all the wasps had died their remains were removed, the plastic bag was detached and the plants were moved outdoors and monitored for six months to detect whether or not there was any gall development. Given that the immature stages are endopahgous and immobile there was no risk from retaining plants outdoors during this phase of the life cycle. The numbers of galls formed, along with their dimensions, were recorded.

Surveys in South Africa and Australia - When possible, each of the species, or close relatives thereof, on which eggs were laid in quarantine was surveyed to determine whether the wasps induced galls on these species in South Africa and Australia, in the field. In South Africa, plants were surveyed in the Western Cape, Cape Region, where *A. longifolia* used to be very abundant and still exists at somewhat lower levels. *Vitis vinifera* L. is widely cultivated in the region and *A. melanoxylon* is common but *Cytisus striatus* (Hill) Rothm is not present at all. Another species of a former *Cytisus* (*C. monspessulanus* L. = *Teline monspessulana* (L.) K. Koch.) and the closely related *Spartium junceum* L. (spanish broom) were surveyed for galls. For each plant species, sites were selected where the plant species being surveyed was growing in close proximity (< 25 m) to *A. longifolia* plants with galls. In Australia, plants were surveyed in New South Wales, Wollongong. *Acacia* *longifolia, A. melanoxylon, T. monspessulana* and *V. vinifera* were all surveyed but only *A. melanoxylon* was found in close proximity to *A. longifolia*.

For each sample, 10 plants of the non-target test species and 10 *A. longifolia* plants were randomly selected and observations were made to determine whether the plants had galls by searching for at least 15 min. In species where galls were located, 10 branches were randomly selected and the terminal 70 cm of each was examined to record the number of *T. acaciaelongifoliae* galls per branch. The observations were made during November (2008 and 2009) and March (2009), when the galls were completely formed and easy to detect (both during and after emergence of the adult wasps).

Statistical analyses - Time spent on each behavioral element was recorded in seconds and mean values per species were calculated and compared between species using a General Liner Model (GLM), with a between-subject design One-way ANOVA. The bud dissections were used to calculate the percentage of both buds and branches with eggs for each plant species. The quantity of eggs per branch was also recorded and compared using One-way ANOVA. In paired-choice tests, the One-way ANOVA was used to compare, separately, results of each pair of species. In no-choice tests, the buds were categorized according to size, and the mean number of eggs laid on each bud category on each plant species, was compared using a GLM with a between-subject design Factorial ANOVA. Differences between means were compared with Tukey's test at 5% level of significance. When needed, data were log transformed to fulfill ANOVA assumptions. STATISTICA 6.0 (StatSoft, Inc. 2001, www.statsoft.com) was used for the statistical analysis.

RESULTS

No-choice tests – observations of the oviposition behavior of *T. acaciaelongifoliae*

Behavioral elements did not occur in any particular sequence. Some behavioral elements ("Active on plant", "Off plant" and "Stationary on plant") were observed on almost all of the plant species that were tested (Table 6.2). The wasps were observed exploring the buds of only 17 species (nine species had wasps stationary on the buds and 12 species had wasps that were active on the buds), with no significant differences between species (Table 6.2). Ovipositional probing was noted on the target species *A. longifolia* and six non-target species, including all the species where eggs were later detected. With the exception of *A. longifolia, A. melanoxylon* and *C. striatus,* this behavior was observed only once on each plant species.

Wasps were observed on the buds for ca. 3% of the total time of observations on all the plants (123 h), and spent more time off the plants (ca. 53% of the total time) followed by being stationary on the plants (32.5% of the total time). On species where egg deposition was confirmed (Figure 6.3) the wasps spent more time on the buds, mainly on buds of *A. longifolia, A. melanoxylon* and *C. striatus*.

Table 6.2 Time (mean (SE) in sec.), that *Trichilogaster acaciaelongifoliae* spent on each behavioral element* of each plant species in no-choice tests.

species	Off Plant	SE	Tukey	Active on plant	SE	Tukey	Stationary on plant	SE	Tukey	Active on buds	SE	Tukey	Stationary on bud	SE	Tukey	Probing	SE	Tukey
Acacia longifolia	644	(107.1)	ab	148.2	(25.4)	ab	191.6	(72.8)	abc	36.4	(36.4)	a	115.4	(68.0)	a	64.3	(47.4)	a
Acacia melanoxylon	574	(174.5)	ab	115.9	(37.8)	ab	162.8	(102.5)	abc	105.1	(90.8)	a	231.1	(119.8)	a	10.9	(7.4)	a
Arbutus unedo	634	(153.4)	ab	45.4	(14.1)	a	521.0	(148.6)	abc	--	--		--	--		--	--	
Ceratonia siliqua	547	(108.9)	ab	125.4	(36.6)	ab	527.8	(111.1)	abc	--	--		--	--		--	--	
Cistus psilosepalus	612	(116.3)	ab	236.5	(62.2)	ab	351.8	(102.8)	abc	--	--		--	--		--	--	
Citrus sinensis	557	(159.3)	ab	168.4	(55.5)	ab	475.0	(162.6)	abc	--	--		--	--		--	--	
Corema album	450	(105.3)	ab	253.7	(76.6)	ab	481.0	(120.4)	abc	--	--		15.5	(15.5)	a	--	--	
Cytisus striatus	718	(131.8)	ab	67.8	(19.7)	ab	47.1	(17.6)	ab	--	--		270.6	(128.3)	a	96.7	(96.7)	a
Erica scoparia	782	(115.3)	ab	121.3	(47.7)	ab	254.0	(128.7)	abc	--	--		--	--		42.8	(42.8)	a
Eucalyptus globulus	599	(142.1)	ab	131.3	(68.2)	ab	470.2	(161.7)	abc	--	--		--	--		--	--	
Genista falcata	821	(158.2)	ab	95.5	(43.3)	ab	279.2	(121.2)	abc	4.7	(4.7)	a	--	--		--	--	
Laurus nobilis	417	(156.8)	ab	83.3	(42.3)	ab	596.0	(163.8)	abc	46.2	(46.2)	a	--	--		57.4	(57.4)	a
Lavandula luisierii	646	(138.3)	ab	66.4	(25.5)	ab	487.1	(135.2)	abc	--	--		--	--		--	--	
Malus domestica	702	(157.9)	ab	280.6	(153.7)	ab	118.5	(45.2)	abc	--	--		99.0	(99.0)	a	--	--	
Medicago marina	1062	(66.7)	b	118.6	(59.5)	ab	16.9	(8.4)	a	2.1	(2.1)	a	--	--		--	--	
Myrica faya	543	(147.9)	ab	58.1	(24.9)	a	577.6	(147.5)	abc	21.3	(21.3)	a	--	--		--	--	
Phaseolus vulgaris	636	(177.3)	ab	69.7	(33.0)	a	494.5	(188.4)	abc	--	--		--	--		--	--	
Phillyrea angustifolia	783	(98.1)	ab	119.1	(36.3)	ab	213.8	(67.4)	abc	84.2	(55.3)	a	--	--		--	--	
Pinus pinaster	431	(144.7)	ab	239.6	(85.6)	ab	522.9	(145.3)	abc	3.8	(3.8)	a	2.3	(2.3)	a	--	--	
Pistacia lentiscus	704	(121.7)	ab	117.6	(45.7)	ab	378.8	(109.0)	abc	--	--		--	--		--	--	
Pisum sativum	615	(197.7)	ab	214.0	(123.2)	ab	371.4	(208.3)	abc	--	--		--	--		--	--	
Polygala vulgaris	904	(168.8)	ab	40.1	(20.1)	a	255.9	(161.8)	abc	--	--		--	--		--	--	
Prunus lusitanica	925	(64.1)	ab	104.7	(24.0)	ab	170.2	(52.8)	abc	--	--		--	--		--	--	
Prunus persica	517	(157.8)	ab	192.1	(94.6)	ab	491.2	(141.3)	abc	--	--		--	--		--	--	
Pseudotsuga menziesii	286	(124.5)	a	220.5	(69.9)	ab	693.8	(143.7)	bc	--	--		--	--		--	--	
Pyrus communis	1168	(19.2)	ab	16.3	(10.0)	ab	6.8	(6.8)	a	9.0	(9.0)	a	--	--		--	--	
Quercus faginea	394	(135.0)	ab	118.4	(35.5)	ab	672.0	(137.6)	bc	6.3	(6.3)	a	6.6	(6.6)	a	3.2	(3.2)	a
Quercus lusitanica	741	(104.9)	ab	55.6	(22.8)	ab	403.1	(93.6)	abc	--	--		--	--		--	--	
Quercus pyrenaica	379	(135.6)	ab	55.3	(19.9)	a	766.2	(137.7)	b	--	--		--	--		--	--	
Quercus robur	654	(106.8)	ab	370.5	(108.1)	ab	175.4	(75.0)	abc	--	--		--	--		--	--	
Quercus rotundifolia	671	(122.8)	ab	109.5	(19.4)	ab	420.0	(130.1)	abc	--	--		--	--		--	--	
Quercus suber	625	(131.5)	ab	95.6	(24.0)	ab	479.4	(125.3)	abc	--	--		--	--		--	--	
Quercus x coutinhoi	773	(109.2)	ab	212.0	(77.8)	ab	214.7	(70.4)	abc	--	--		--	--		--	--	
Rhamnus alaternus	604	(168.6)	ab	133.7	(85.6)	ab	449.7	(160.8)	abc	12.9	(12.9)	a	--	--		--	--	
Salix atrocinerea	586	(104.9)	ab	99.3	(33.5)	ab	515.3	(100.1)	abc	--	--		--	--		--	--	
Stauracanthus genistoides	755	(160.3)	ab	160.5	(116.6)	ab	284.1	(140.1)	abc	--	--		--	--		--	--	
Ulex parviflorus	425	(119.5)	ab	142.0	(33.2)	ab	558.6	(130.0)	abc	--	--		74.3	(54.1)	a	--	--	
Ulmus procera	367	(153.5)	ab	201.4	(76.7)	ab	583.8	(161.1)	abc	--	--		47.4	(31.5)	a	--	--	
Viburnum tinus	621	(129.4)	ab	333.3	(48.1)	b	244.7	(95.2)	abc	--	--		--	--		--	--	
Vicia faba	469	(175.5)	ab	228.8	(101.4)	ab	502.1	(155.4)	abc	--	--		--	--		--	--	
Vitis vinifera	572	(150.9)	ab	63.6	(24.1)	ab	544.1	(160.9)	abc	17.5	(17.5)	a	--	--		2.5	(2.5)	a
mean time (sec.)	632.0	(132.2)		142.2	(52.7)		389.5	(118.3)		29.1	(25.5)		95.8	(58.3)		39.7	(36.8)	
total time (sec.)	25910.4			5829.3			15969.9			349.4			862.2			277.7		
% time spent/behaviour*	52.7			11.8			32.5			0.7			1.8			0.6		
no. species/behaviour	41.0			41.0			41.0			12.0			9.0			7.0		

* **Active on plant/buds**, wasps walking on the branches or leafs/buds with their antennae not in contact with the bud. **Stationary on plant/ buds**, wasps stationary on the branches or leafs/ buds. **Probing**, wasps with the ovipositor inserted into the bud (not always associated with egg deposition). **Off plant**, wasps exploring the cage without contacting the plant. "—" Denotes behavioral element was not registered on the species.

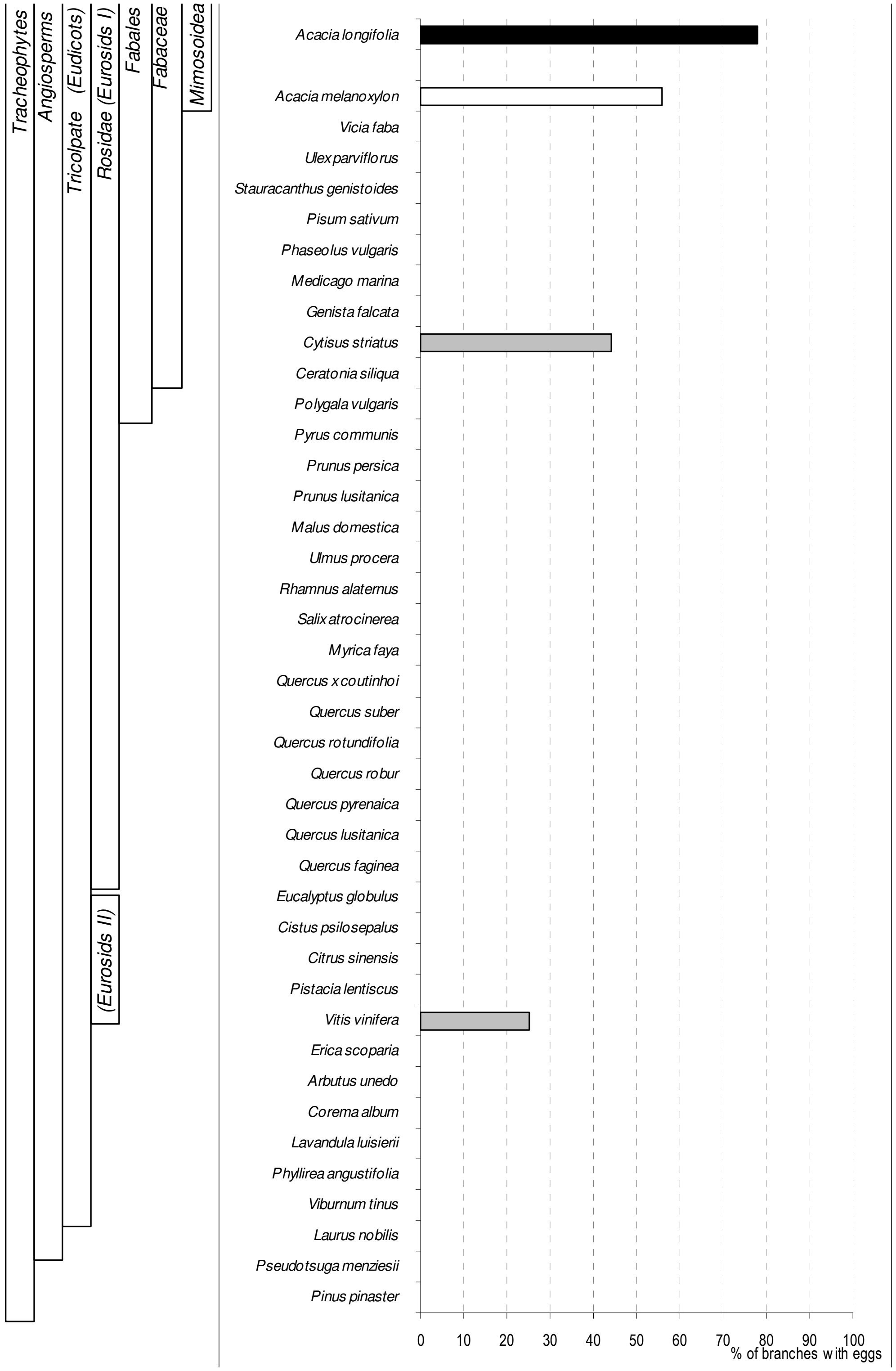

Figure 6.3 Percentage of branches where eggs of *T. acaciaelongifoliae* were detected amongst all of the species tested. The species are ordered (top to the base) according to phylogenetic closeness to *A. longifolia.*

No-choice tests – dissection of plant species buds to detect *T. acaciaelongifoliae* eggs

Dissection of the buds of *Erica scoparia*, *Quercus faginea* and *Laurus nobilis* L., all off the taxonomic order *Fabales* (Figure 6.3), showed that although the wasps had been observed probing the buds of these species (Table 6.2), no eggs were laid on any of these plants. Of the nine females placed on each plant species, seven laid eggs on the target species, *A. longifolia,* five laid on *A. melanoxylon*, four on *C. striatus* and two laid eggs on *V. vinifera*. Eggs were laid on 21.8% of the buds of *C. striatus* that were exposed to the wasps while on *A. melanoxylon* only about 10% of buds had eggs. On *V. vinifera* only 4.3% of the buds had eggs whereas on *A. longifolia* eggs were laid in 31.8% of the buds. On *C. striatus* and *A. melanoxylon* (which were included in the test-list because of their close relationship to *A. longifolia*), eggs were laid within the bud tissues as happens on *A. longifolia.* In the case of *V. vinifera* eggs were laid on the protective, pubescent outer layer of the buds and not within the bud tissues. The number of eggs per branch varied with plant species ($F_{3,32} = 4.182$, $p = 0.013$), with significantly more eggs laid per branch on *A. longifolia* than on *V. vinifera* and *A. melanoxylon* while the numbers laid on *C. striatus* were intermittent between *A. longifolia* and the other two species, and not significantly different from any of the others (Figure 6.4).

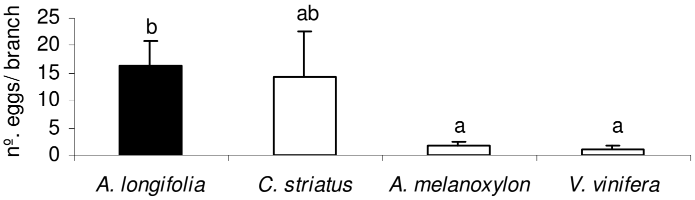

Figure 6.4 Number of eggs (mean + SE per branch) laid on *Acacia longifolia* and the three non-target plant species where eggs were detected (Tukey test, P < 0.05).

Trichilogaster acaciaelongifoliae showed a clear 'preference' for laying eggs on buds that were smaller than 3 mm (Figure 6.5).

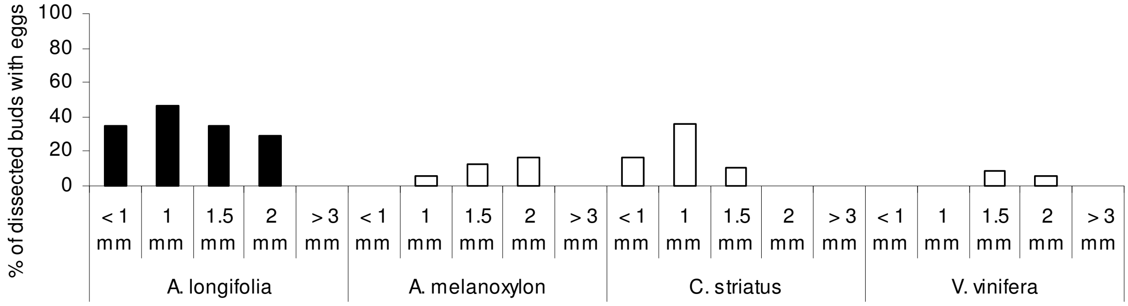

Figure 6.5 Percentage of different-sized buds that had eggs of *Trichilogaster acaciaelongifoliae* on four different plant species (n = 9 females exposed on each plant species).

On *C. striatus* most eggs were found in the smallest buds (<1-1.5 mm) while on *V. vinifera* the eggs were found predominantly on larger buds (1.5 - 2 mm). The target species *A. longifolia* had eggs in a wider range of bud sizes up to 3 mm with uniform pattern of around 30% and 40% of the buds in each size class having eggs. On *A. melanoxylon* the eggs were found mostly in the intermediate sized buds (1-2 mm). The pattern of bud use was also reflected in the numbers of eggs which were deposited in the different sizes of buds (Figure 6.6). For each of the four plant species, buds generally decreased in size from the proximal to the terminal portion of the branches.

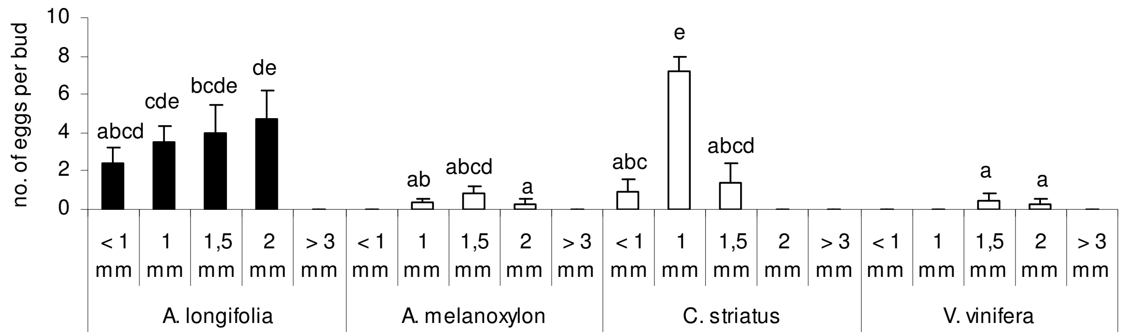

Figure 6.6 Number (mean + SE) of eggs of *Trichilogaster acaciaelongifoliae* in different sized buds of four plant species (*A. longifolia* and the three non-target species where eggs were detected). Columns with the same letters are not significantly different from each other (Tukey test, P < 0.05).

Paired-choice tests – observations of *T. acaciaelongifoliae* behavior

When in contact with both species of each paired-choice test *T. acaciaelongifoliae* spent significantly more time in contact with the target *A. longifolia* than with *C. striatus* and *A. melanoxylon* (Table 6.3).

Table 6.3 Time (mean + SE, in sec.), that *Trichilogaster acaciaelongifoliae* spent on each behavioral element of each plant species in paired-choice tests.

pair of species	n	df	Active on plant	Stationary on plant	Active on buds	Stationary on buds	Total Time on plant	Off plants
A. longifolia *C. striatus*	9	1,16	81.3 (18.8) 69.0 (48.1)	299.2 (151.2) 58.0 (38.9)	9.6 (6.6) 0.0	306.0 (146.3) 56.1 (39.4)	**696.8 (175.3)** **183.1 (100.5)**	320.1 (130.8)
			F = 0.06; *p = 0.81*	*F = 3.30;* *p = 0.09*	*	*F = 2.73;* *p = 0.12*	***F = 6.46;*** ***p = 0.02***	
A. longifolia *V. vinifera*	9	1,16	122.9 (39.0) 39.8 (30.7)	201.2 (109.4) 284.7 (97.2)	0.0 0.0	124.4 (88.2) 0.0	448.6 (146.3) 324.4 (107.4)	427.0 (111.5)
			F = 2.80; *p = 0.11*	*F = 0.32;* *p = 0.58*		*	*F = 0.47;* *p = 0.50*	
A. longifolia *A. melanoxylon*	9	1,16	**71.1 (17.9)** **27.0 (25.3)**	**261.1 (113.7)** **26.8 (26.8)**	0.0 0.0	**408.1 (165.5)** **0.8 (0.8)**	**740.3 (181.6)** **54.6 (52.8)**	405.1 (165.0)
			F = 12.17; ***p = 0.003***	***F = 18.76;*** ***p = 0.0005***		***F = 5.26;*** ***p = 0.04***	***F = 13.14;*** ***p = 0.002***	

* Statistical analysis was not performed in situations where the wasps did not select both species. Significant differences are **in bold.**

The wasps were observed contacting the buds of the non-target species very briefly. For the pair *A. longifolia* - *A. melanoxylon* the wasps spent significantly more time in *A. longifolia* whatever the behavioral element was.

Paired-choice tests - dissection of plant buds to detect *T. acaciaelongifoliae* eggs

A higher percentage of *A. longifolia* branches had eggs, reaching 80% in the pair *C. striatus, A. longifolia,* than any of the non-target species where the maximum value was 40% on *C. striatus* (Figure 6.7). When able to choose the target, no wasps selected *V. vinifera*. A similar trend was observed in the quantity of buds with eggs, with fewer buds infected per branch of the non-target species than *A. longifolia* in all paired-choice tests: on average, 11.3% of buds on branches of *C. striatus* had eggs, while on *A. melanoxylon* only 5.6% of the buds had eggs. On *A. longifolia* eggs were laid in 52.4%, 20.9% and 38.8% of the buds in paired choice tests with *C. striatus, A. melanoxylon* and *V. vinifera*, respectively.

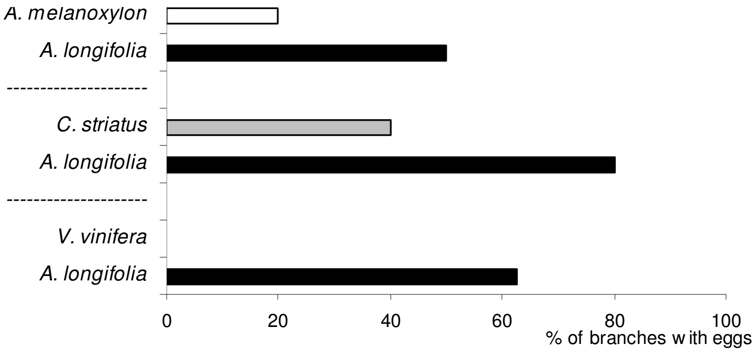

Figure 6.7 Percentage of branches of the target and non-target species on paired-choice tests where *Trichilogaster acaciaelongifoliae* laid eggs.

In paired-choice oviposition tests, *T. acaciaelongifoliae* laid significantly more eggs per *A. longifolia* branch than in any of the other three species (Figure 6.8) (*V. vinifera* $F_{1,14} = 5.326$, $p = 0.037$, *C. striatus* $F_{1,16} = 12.355$, $p = 0.003$, *A. melanoxylon* $F_{1,14} = 5.423$, $p = 0.036$).

The pattern of egg distribution relative to bud size was similar to the one observed in no-choice tests, with eggs occurring in all sizes of *A. longifolia* buds (Figure 6.9). Some eggs were detected in larger buds than in the no-choice tests. The larger buds on *A. longifolia* included flower buds with the inflorescence spike already clearly developed.

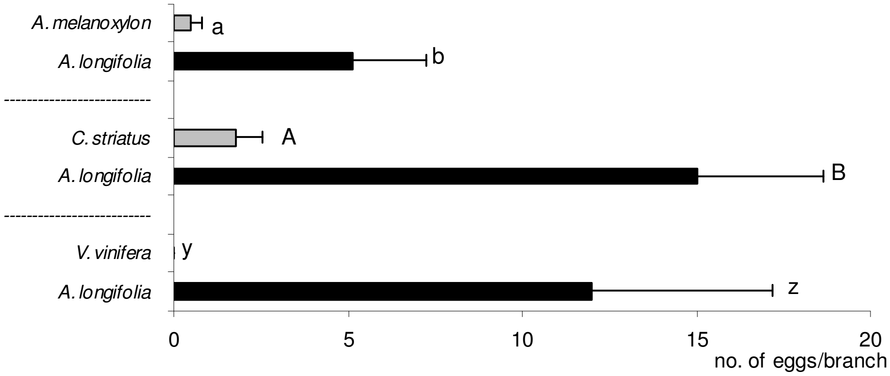

Figure 6.8 Mean number of eggs laid by *Trichilogaster acaciaelongifoliae* females on *Acacia longifolia* (target) and on non-target species in paired-choice tests. Species of each pair were compared with 1-Way ANOVA.

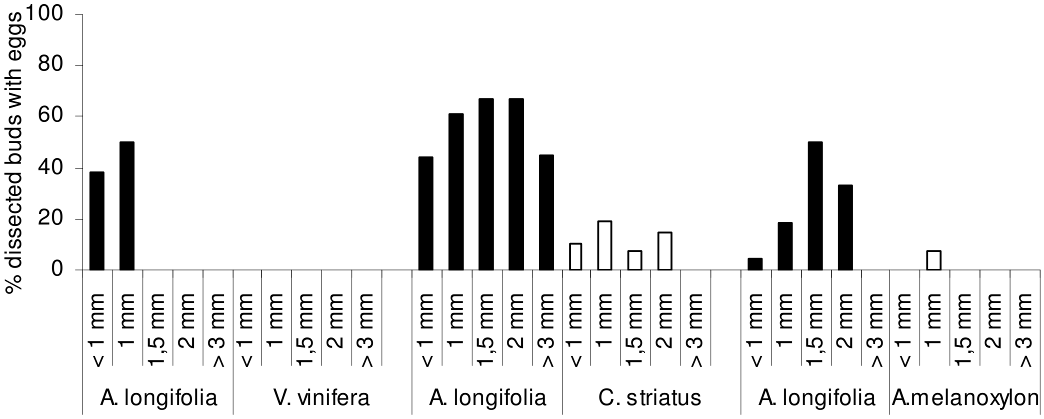

Figure 6.9 Percentages of different-sized buds that had eggs of *Trichilogaster acaciaelongifoliae* during paired-choice tests with three non-target species.

Gall induction on potted plants

After exposure to *T. acaciaelongifoliae*, galls only developed on potted *A. longifolia* plants. Three of the six potted *A. longifolia* (*i.e.,* 50%) developed galls in low numbers. One plant had three galls which were 2, 4 and 6 mm in diameter, and the other two plants had one gall each, which were 7 and 9 mm in diameter. No galls developed on either of the other two species. Although the plants were healthy when presented to the wasps, some perished during the subsequent monitoring period. Nevertheless, galls were clearly visible within two months of exposure to the wasps and all of the plants survived for that length of time.

Surveys in South Africa and Australia

In areas where *T. acaciaelongifoliae* has open access to the environment, field surveys revealed that only *A. longifolia* had galls of *T. acaciaelongifoliae* developing on its branches and that galls were more abundant in South Africa, where the wasp is introduced, than in Australia, the native home of the wasps (Figure 6.10).

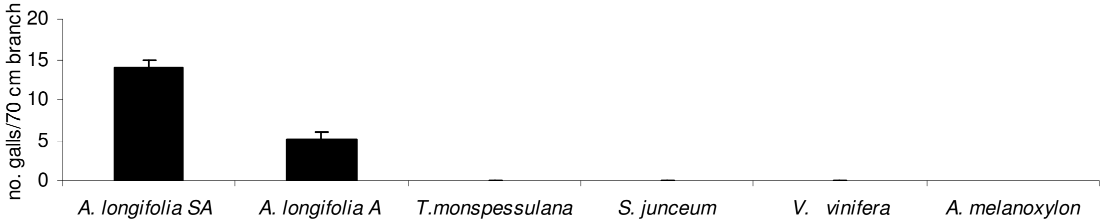

Figure 6.10 The abundance of galls of *Trichilogaster acaciaelongifoliae*, on the terminal 70 cm of branches, on five plant species including the target, *Acacia longifolia,* in South Africa (SA) (Western Cape) and Australia (A) (New South Wales). The non-target species included two species (*Vitis vinifera* and *Acacia melanoxylon*) on which the wasps laid eggs during no-choice tests, and two species closely related to *Cytisus striatus (T. monspessulana* and *S. junceum)*. Non-target species were sampled in SA and A, except for *Spartium junceum* which was only sampled in in SA.

DISCUSSION

Even though international regulations are based on risk analysis schemes (EPPO, 2009a), when deciding whether to use biological control as part of a management strategy against invasive alien plants, the risks of releasing agents must be weighed against the potential costs and benefits, including, critically in this case, whether or not suppression of *A. longifolia* in Portugal would be possible and affordable without the intervention of biological control. The impacts of *A. longifolia* invasions on biodiversity and conservation in Portugal are well documented (Marchante *et al.*, 2003, 2004, 2008a,b, 2009; chapter 2). In addition, management interventions practiced thus far have failed in the long term because the invader has resurged from accumulated seed banks (chapter 3; Marchante, Freitas & Hoffmann, 2010a) and financial constraints have frequently prevented follow up control. Biological control is usually considered as the most cost effective and environmentally-sound form of weed control (Holden, Fowler & Schroeder, 1992). Based on precedents in South Africa (Dennill & Donnelly, 1991; Dennill *et al.*, 1999), it is highly likely that, were *T. acaciaelongifoliae* to be released in Portugal, it would significantly reduce the invasiveness of *A. longifolia.*

What are the chances that *T. acaciaelongifoliae* will "attack" non-target species?

In Petri dishes, *T. acaciaelongifoliae* laid eggs on buds of three non-target plants. On two of these, *C. striatus* and *A. melanoxylon* (which were included in the test-list because of their close phylogenetic relationship to *A. longifolia*), eggs were laid within the bud tissues as happens on *A. longifolia.* In the case of *V. vinifera* eggs were only found on three buds and in each case the eggs were laid on the protective, pubescent outer layer of the buds and not within the bud tissues, *i.e.*, in a localization inappropriate to induce the development of the gall.

Oviposition on *A. melanoxylon* was no surprise because, besides being a close relative of *A. longifolia, T. acaciaelongifoliae* was observed to probe its buds during host specificity testing in South Africa (Van den Berg, 1980). Besides, the species is known to serve, though very infrequently, as a 'subsidiary' host for *T. acaciaelongifoliae* in South Africa, in situations where *A. melanoxylon* grows in close proximity to heavily-galled *A. longifolia* plants (Dennill & Donnelly, 1991). Of the three plant species under consideration, the situation with *A. melanoxylon* is of least concern in the exercise of considering the introduction of *T. acaciaelongifoliae* into Portugal, as it has no economic value in Portugal and is currently considered invasive under Portuguese legislation which prohibits its use and encourages its control (Ministério do Ambiente, 1999). Galling on this species would not be problematic and would most likely be infrequent and sporadic if ever.

Infliction of damage by *T. acaciaelongifoliae* on either *C. striatus* or *V. vinifera* would not be acceptable. *Cytisus striatus* is a native species in Portugal where it, and several of its congeners, are popular ornamental plants while *V. vinifera* has enormous economic value. Oviposition on these two species, and especially *V. vinifera*, was quite unexpected. A convincing line of evidence that *T. acaciaelongifoliae* will not associate with either of these species under natural conditions in Portugal comes from the trials in which no galls developed on potted plants of either *C. striatus* or *V. vinifera* after exposure to *T. acaciaelongifoliae*, showing that these species do not meet the requirements of the immature stages of the wasps. Additional evidence that *V. vinifera* will not be affected by the wasp comes from paired-choice tests (where *V. vinifera* was rejected) and from field observations; in South Africa where many different cultivars of vines are frequently cultivated in close proximity to galled *A. longifolia* plants no records have ever been made of galls on the vines, either historically or during the surveys which constituted part of this study. Similarly, several species of *Cytisus*, and the closely-related *Genista*, are naturalized in South Africa (Henderson, 1995) and Australia (Groves, Boden & Lonsdale, 2005), with some being invasive. They commonly grow in areas where *A. longifolia* occurs. In spite of this prolonged association, no *T. acaciaelongifoliae* galls have ever been reported on any of these plants and no galls were encountered during the field surveys that formed part of this study.

For all the above reasons, the observations of *T. acaciaelongifoliae* laying eggs on buds of *V. vinifera* and *C. striatus* in Petri dishes were in every likelihood laboratory artifacts, induced by the confined conditions and by the lack of suitable host plant material being available to the females. In paired-choice tests, the wasp never laid eggs on *V. vinifera*, and both the percentage of buds infected with eggs and the number of eggs per bud on *C. striatus* were markedly reduced. Confinement in cages is well known to disrupt normal behavioral (including olfactory and gustatory) responses of herbivorous insects and induces them to develop on a much wider range of plants (termed the physiological host range) than they would do naturally (Marohasy, 1998; Heard, 2000; Van Klinken, 2000; Withers, Barton Browne & Stanley, 2000; Sheppard, van Klinken & Heard, 2005). Such a situation arose during the early stages of the only other biological control program against an invasive plant in Europe. In that case, a psyllid, *Aphalara itadori*, was being considered as a possible agent for biological control of *Fallopia japonica* in the UK (Shaw, Bryner & Tanner, 2009). Despite the ambiguous results of laboratory tests, the psyllid was approved for release early in 2010 (Shaw, Bryner & Tanner, 2009). Another example, is the oviposition by *Evippe* sp. (Lepidoptera: Gelechidae), in cages, on five non-target species (all within the same family as the target weed) with little subsequent larval development (Van Klinken & Heard, 2000). The discrepancy between female choice and larval development and survival led Van Klinken and Heard (2000) to conclude that the moth would be confined to the target weed in a field situation and should therefore be released as a biological control agent.

Parasitism of galls, unsuitable climate and crossing hemispheres – are there reasons to be concerned?

Besides the need for assurances that *T. acaciaelongifoliae* will be restricted to *A. longifolia,* the possible acquisition of natural enemies, unsuitable climatic conditions or translocation from the southern to the northern hemisphere may potentially limit its effectiveness in Portugal or even prevent its establishment altogether.

In South Africa, *A. longifolia* trees are generally more heavily galled than they are in Australia, where *T. acaciaelongifoliae* suffers high levels of parasitism and has to compete with other bud-feeding insects (Neser, 1984). This discrepancy persists even though *T. acaciaelongifoliae* is attacked by several native parasitoid species in South Africa (Hill & Hulley, 1995; Manongi & Hoffmann, 1995; Seymour & Veldtman, 2010). There is no reason to expect that indigenous hymenopterous parasitoids will not utilize the larval and pupal stages of *T. acaciaelongifoliae* in Portugal (Noyes, 2003) but, because there are no ecological analogues (*i.e.,* gall forming insects on acacias in Portugal), the impact of parasitoids, both directly on the efficacy of *T. acaciaelongifoliae* (Paynter *et al.*, 2010) and indirectly on the local food webs are likely to be trivial.

In South Africa, *T. acaciaelongifoliae* is reported to be most effective in warm temperate areas. In terms of the Köppen-Geiger climate classification system (Kottek *et al.*, 2006), these areas are grouped as Csb (dry summers) and Cfb (humid summers), with a threshold temperature of > 10ºC for at least four months of the year and a mean temperature for the hottest month of ≥ 22º C; *i.e.,* areas that are climatically similar to areas where *T. acaciaelongifoliae* was originally collected in Australia (Cfb) (Dennill, 1987). Most of the Portuguese coastal region (except for the southern extremity of the country) and the continental north of Portugal, extending into northwestern Spain is classified as Csb (Kottek *et al.*, 2006). In Portugal, *A. longifolia* is invasive mainly in the coastal regions, extending to Galicia, Spain, (Elorza, Sánchez & Vesperinas, 2004), where climatic conditions would be favorable for the development and survival of *T. acaciaelongifoliae*. Thus, there should be no concerns that climatic-mismatching would dampen the performance of the wasp, were it to be released in Portugal.

In moving *T. acaciaelongifoliae* from South Africa to Portugal, consideration needs to be given to the asynchronous phenology of the host plant in the southern and northern hemispheres. Female wasps moved from South Africa in October/November when the adults are most abundant will be faced with host plants in late-autumn stages in Portugal when most of their buds will be larger than the prefered size (>2 mm in length) for egg deposition. Although *T. acaciaelongifoliae* may select some larger buds, at that time of year there would also be some smaller buds on the plants which should enable the wasps to establish founder populations. Alternatively, lower numbers of *T. acaciaelongifoliae* females could be collected in South Africa earlier in the year (N. Dorchin, *personal communication*) and shipped to Portugal when the *A. longifolia* plants would be in a more suitable phenological stage. Either way, provided enough wasps are released over a sufficiently long period of time, some females should oviposit successfully and produce founding and then burgeoning populations of adults synchronized with the phenology of *A. longifolia* in Portugal.

Should *T. acaciaelongifoliae* be used as part of a management strategy to control *A. longifolia* in Portugal?

All indications are that there are no substantive reasons not to release *T. acaciaelongifoliae* in Portugal and thereby alleviate the overwhelming negative impacts of *A. longifolia*. The extremely slight risk that the wasps might lay some eggs on plants other than *A. longifolia,* the potential indirect non-target effects due to parasitoids acquisition, and the minimal consequences thereof, are more than offset by the substantial benefits that will accrue if the project succeeds. Biological control is the only way to prevent an escalation in levels of irreversible damage that *A. longifolia* will inevitably inflict on the ecology and biodiversity of whole communities of native organisms in Portugal, and further afield in Europe.

Further steps to be taken before release….

The release of the wasp *T. acaciaelongifoliae* is the ultimate goal of the research project and permissions to do so will have to conform to a series of legal procedures. Any request for release of an exotic biocontrol agent against an invasive plant species will be the first in Portugal (and only the second in Europe). The regulatory framework to follow is not yet well "established". Nevertheless, considering the framework followed in UK (Djeddour & Shaw, 2010), and which almost certainly will have created precedents, the permit to release *T. acaciaelongifoliae* will have to involve the preparation of a pest risk analysis (PRA) as this organism is likely to cause injury to a plant (*A. longifolia*) within the PRA area. Part of the information needed to the PRA has been gathered previously to support the permission to bring the agent into quarantine. *Acacia longifolia* is considered naturalized in Spain, France and Italy, though it is only considered invasive in Spain (Elorza, Sánchez & Vesperinas, 2004; Celesti-Grapow *et al.*, 2009). Nevertheless, there are other regions in Europe which have Csb or Cfb climates (Kottek *et al.*, 2006), so the potential for the wasp to spread across political boarders will need to be addressed in the PRA. PRA will have to be included in an application to Portuguese Institute for Conservation of Nature and Biodiversity (ICNB, from Ministério do Ambiente e do Ordenamento do Território - MAOT) with the intention to provide adequate information for the decision to be made to: 1) free *T. acaciaelongifoliae* from the restrictions of the Decreto-Lei n.º 565/99, under which it is being held, by default once is not listed, as a "forbidden non-indigenous species", and 2) to allow its intentional release in the wild. Possibly, the application will have to be reviewed by a Standing Committee, as well as by experts. Other European Member States (MS), particularly in neighbouring MS and those in the same eco-climatic zones should be informed of the intention to release and allowed to comment.

According to the EPPO decision-support scheme (EPPO, 2009a), the PRA will include a brief initiation (stage 1 – Appendix 6.1) and two major sections that aim to perform a risk assessment (stage 2 - Appendix 6.2): Section A. categorizes the "pest" and is basically an assessment in the form of a binary decision tree, constructed from a sequence of questions based largely on decision points; and Section B. aims to assess the probability of introduction and spread and of potential economic consequences and is a more detailed assessment that will be performed only if the section A leads to the conclusion that the organism has the characteristics of a quarantine pest.

The risk assessment should lead to a conclusion concerning the level of "risk' presented by the agent, that should inform the decision to continue to phase 3 - the Pest Risk Management (Appendix 6.3) which aims to determine whether the risk is acceptable, and, to identify management options. At a certain stage, which could be before or during the Pest Risk Management phase, a public consultation should take place.

Only if all the abovementioned stage result in a positive conclusion would a final approval, from MAOT be required that will potentially culminate in the agent release. If permission to release is granted, contingency and monitoring plans should be in place before release.

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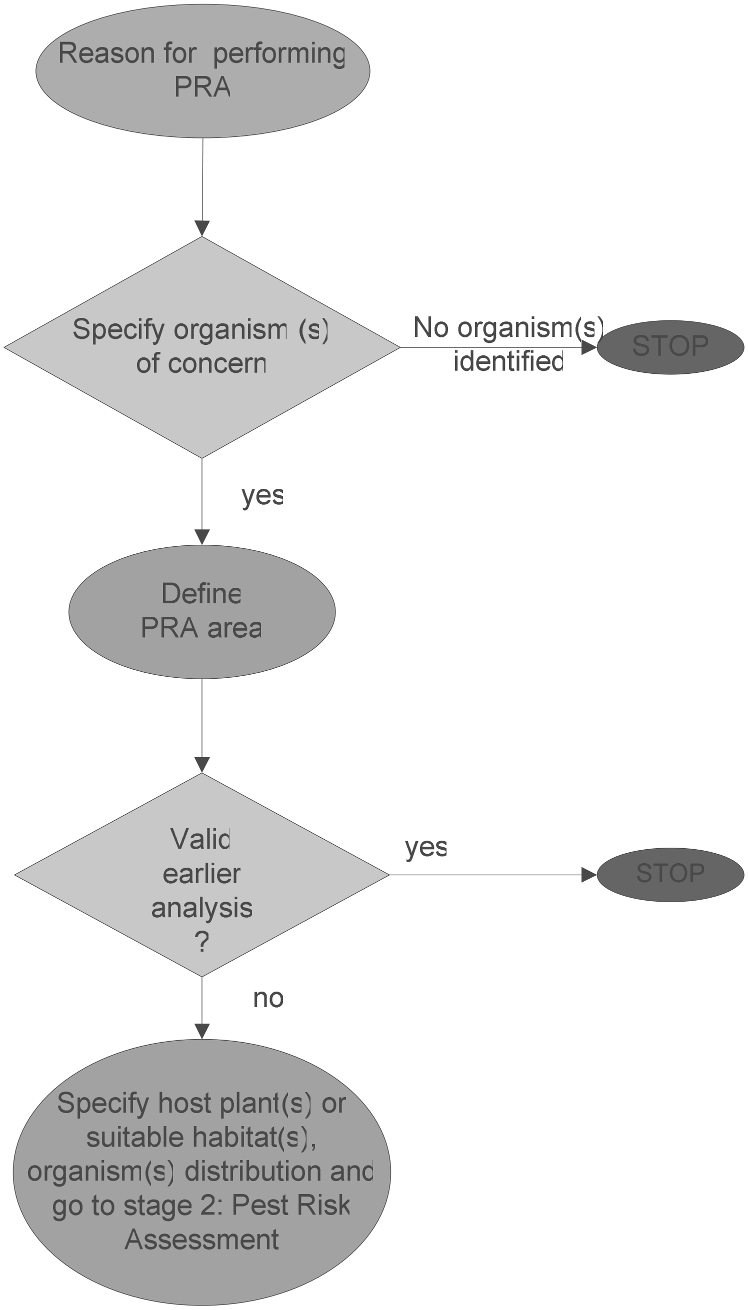
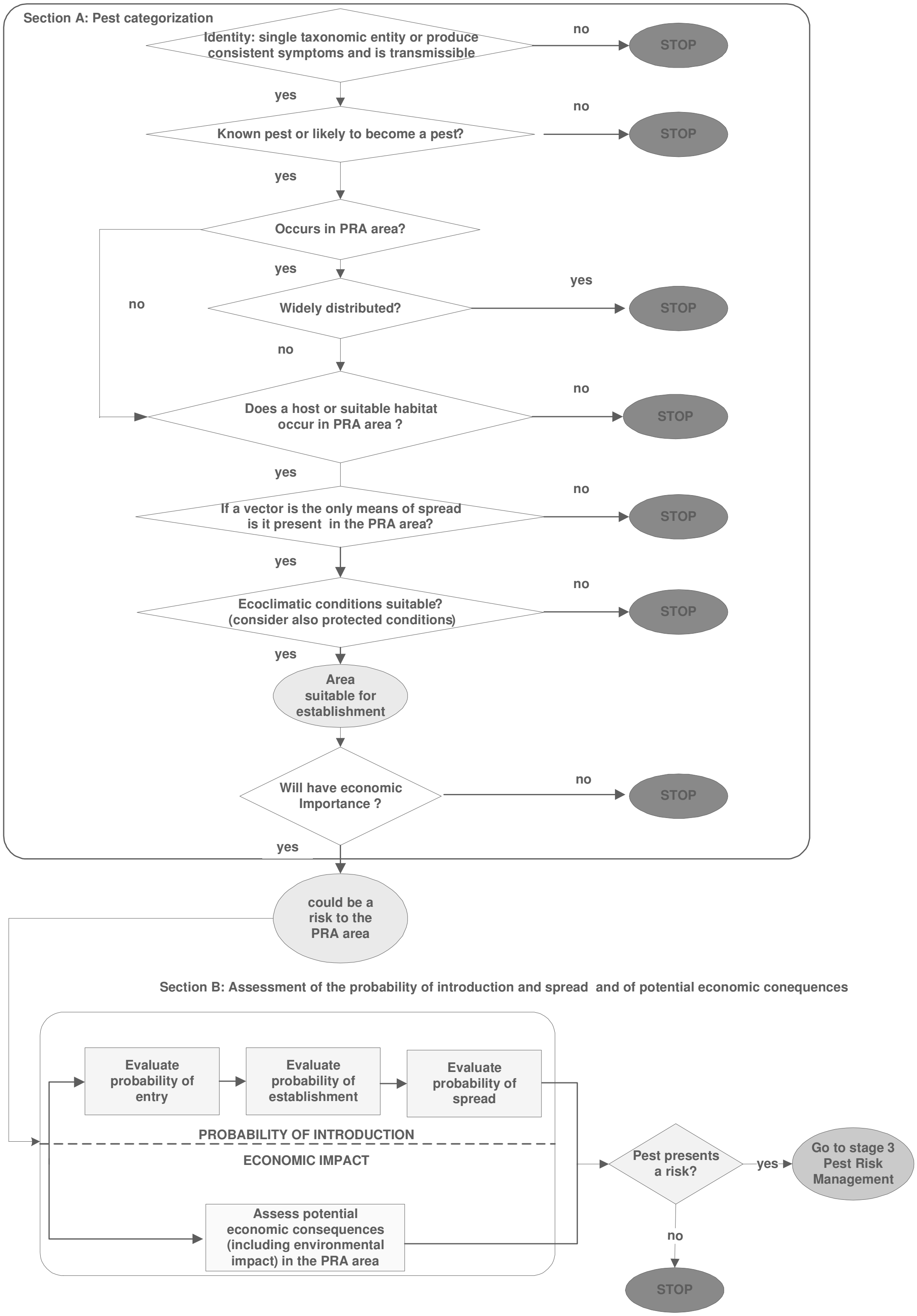
APPENDIX

Appendix 6. 1



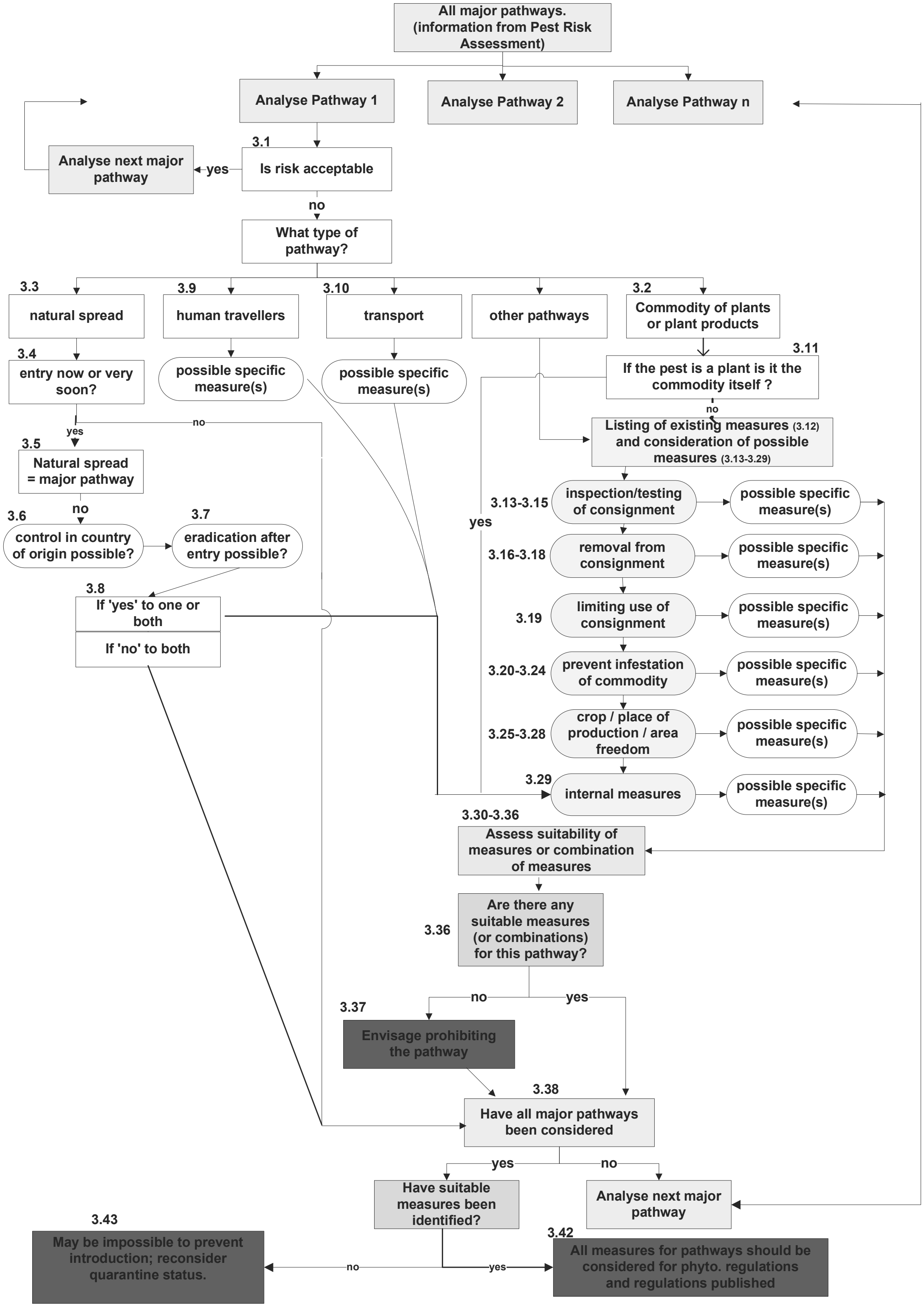
Decision-support scheme for quarantine pests Stage 1 Initiation (source: EPPO (2009b)

Appendix 6. 2



Decision-support scheme for quarantine pests Stage 2 Pest Risk Assessment (source: EPPO (2009b)

Appendix 6. 3



Decision-support scheme for quarantine pests Stage 3 Pest Risk Management (source: EPPO (2009b)

Part III

Chapter 7

General Discussion and Conclusions

Despite the profound impacts of *A. longifolia* invasion on native vegetation (chapter 2), including the composition of seed banks (chapter 4, experiment I), plant species recovery observed in the field (chapter 5) and in soil seed bank studies (chapter 4, experiment II), showed that the dune systems under investigation retained some resilience. Nevertheless, system degradation was striking, increasing with time of invasion (chapter 2). Furthermore, invaded systems become highly vulnerable to reinvasion even if the existing stands of *A. longifolia* are removed due to the persistent seed-banks of *A. longifolia* (chapter 3). The challenge is to find management options that can be sustainable in the long-term. Sustainability is particularly important because funding constraints often preclude continuation of management actions. Therefore, biological control by an agent that targets the reduction of *A. longifolia* seed production, and additionally curtails vegetative growth, can be a valuable tool for management, and this was shown to be a promising opportunity for Portugal (chapter 6).

WHAT HAS CHANGED ON DUNE PLANT COMMUNITIES DUE TO INVASION BY *ACACIA LONGIFOLIA*?

Acacia longifolia is clearly a transformer (sensu Richardson *et al*. (2000)) drastically changing dune landscapes. Changes were detected at several different levels:

- Dune habitat diversity decreased (*i.e.,* the landscape become more homogeneous); plant community structure (*i.e.,* physiognomy and vertical stratification) and species traits were markedly altered; diversity, richness and cover (of species other than *A. longifolia*) declined. These extensive changes are expected to be reflected in changes to ecological webs and ecosystems services.

- Soil seed banks, both due to the presence of invader seeds and lack of several native species, were altered.

- Dominance of native plant species post-clearing showed that the system was still resilient, though resilience capacity decreased, particularly in long-invaded areas, as shown by the lack of some species that are typical of dunes and by the presence of some exotic species (see Box 7.1).

- Some parameters were more affected than others by duration of invasion, but invasion time really made a difference, with the impacts of *A. longifolia* invasion on plant communities increasing over time.

Pristine coastal sand dunes in Portugal are characterized by distinct plant communities/habitats with remarkable floristic and phytocoenotic diversity (Honrado *et al.*, 2006; Silva, 2006) of species that are naturally adapted to nutrient poor sands and plenty of light at soil level (Smith & Huston, 1989). Such communities are becoming increasingly rare with abiotic conditions favourable to their development becoming progressively more altered. Changes in vegetation (Marchante, Marchante & Freitas, 2003; Kutiel, Cohen & Shoshany, 2004; Hellmann *et al.*, 2010), soil functional microbial diversity (Marchante *et al.*, 2008a) and chemical parameters (Marchante *et al.*, 2008b) due to invasions have already been confirmed for these and other Mediterranean dunes.

Box 7.1 Seed banks (chapter 4) and plant recovery in the field (chapter 5): did these studies reach the same conclusions?

Although the overall results of recovery in the field confirmed the findings of seedling emergence, both experiments provided different information and complemented each other. Seedling emergence was the only method revealing seeds of *A. longifolia* in non-invaded areas. Even so, it apparently augmented the levels of degradation under real field conditions, overlooking propagules that migrated from surrounding areas, and missing species that failed to germinate under greenhouse conditions. Despite such limitations, seedling emergence can be useful for management as it allows a quicker assessment of the ecosystem resilience without major clearing efforts. Long-term assessments in field experiments gave a more detailed insight into the recovery potential, which will probably resemble more what will happen after control, but they are more expensive, time-consuming and take longer to provide answers. There was some differences amongst experiments that allowed further conclusions:

1) Recovery in the field revealed more than twice the number of species observed in the seed bank, with differences in traits that were represented. This was probably due to some stimuli operating in the field (*e.g.* passage through animal digestive tracts (Calviño-Cancela, 2004), disturbance (Grubb, 1988)), but not in the greenhouse. Furthermore, long-term field studies allowed succession in the germination of species, as was evident from high turnover rates in conjunction with the immigrating propagules that colonize the gaps created by clearing (Bullock, 2000).

2) Ephemeral exotic species dominated the recently invaded seed bank while they were sparse in the field. This was most likely a sampling artifact (no. of seedling in the seed banks *vs.*% plant cover in the field) that requires cautious interpretation of results, *e.g.* small short-lived exotics, such as *Conyza* spp., were highly abundant as seeds but that did not necessarily translated into a high cover in the field. *Carpobrotus edulis and A. longifolia* required a different analysis. Their seeds were fewer (*A. longifolia*) or scarcely found (*C. edulis*), particularly in long-invaded areas. Nevertheless, having vegetative reproduction, like *C. edulis* (Roiloa *et al.*, 2009), and forming large individuals (*A. longifolia* is a tree up to 8 m and *C. edulis* forms large mats) one propagule/seed may create high cover in the field.

Most of the findings related with vegetation were based on studies which investigated moderate invasion situations and which invariably, as far as we know, were short-term and limited to one invasion period. Long-term studies and assessments of the effects of duration of invasion are important though scarce in invasion ecology (Blossey, 1999; Strayer *et al.*, 2006). Several parts of the present study were long-term and, while corroborating some of the earlier findings, confirmed the impacts of invasion in areas more-densely inva-ded by *A. longifolia*. More importantly, this investigation showed that duration of invasion is relevant as shown by 1) intensification of impacts with time, at vegetation level, 2) impoverishment of seed banks and more contamination by the invader seeds in long-invaded areas, and 3) declines in the resilience of the system, with autogenic recovery decreasing as the invasion persists. Six years after removal of the invader, despite many native species were present, the vegetation did not fully resembled the historical plant communities (Neto, 1993) in either long-invaded or recently invaded areas. The existing stands were actively expanding and showed potential to continue with seeds found several meters from the stands and new invasion foci occurring several hundred meters away; such seeds were possibly dispersed by periodic strong winds (Bullock and Clarke, 2000), rodents (Holmes, 1990), birds (R. Vaz, SJDNR*, personal observation*), or other agents. Results further showed that some changes take years to become measurable, reinforcing the need for long-term studies (see Box 7.2), and emphasised that assessment of impacts and recovery potential, including only parameters expressed by single-measures, can give incomplete pictures of the changes taking place (see Box 7.3).

Box 7.2 Methodology (I): were median/long-term studies worthwhile?

1) Six years after clearing of *A. longifolia* some shifts in recovery of species and patterns were obvious, including substantial increases in the area covered by several native shrubs and the invader itself; this was accompanied by a reduction in total plant cover (excluding *A. longifolia*) due to a substantial decline of some generalist, early coloniser species, which were especially abundant in plots soon after clearing. These shifts would have been missed in a short-time study.

2) Greater changes in recently invaded areas (observed in the five years period), *i.e.,* higher turnover rates, points to an apparent stabilization of impacts in long-invaded areas (after a couple of decades). This would also have been missed in a short-time study.

When compared with equivalent studies on soil *Ecology,* which looked at long-term assessments as well as duration of invasion (Marchante *et al.*, 2008b), this study showed that vegetation reacts faster to invasion than soil. *Vegetatio*n of recently invaded areas showed marked changes while many soil characterristics in the same areas still closely resembled native soils.

Only a few plant species were common to both invaded and non-invaded areas (although seed bank similarity amongst areas was somewhat higher, chapter 4), particularly to long-invaded areas, with reflection in the plant traits that were dissimilar in the different areas. For example, terophytes and generalist species were more abundant in invaded areas, particularly in recently invaded areas, probably as a result of propagule immigration after fire (gap formation), while chamaephytes, geophytes and hemicryptophytes - to which many species characteristic of dunes belong - were almost absent in the understory of these stands and in the seed bank as well. Some natural sand dunes lack persistent, extensive seed banks (Owen *et al.* 2001), partially because perennial species sometimes have alternative means of reproduction (Sternberg *et al.* 2003). Nevertheless, traits/species characteristic of dunes, that were lacking in the seed bank, were also absent in invaded stands, and others were very scarce (possibly close to being excluded) confirming their possible local exclusion. Even so, this does not imply that those species are extinct at a larger scale, a trend that has been similalrly found in other studies (Jäger, Kowarik & Tye, 2009).

Impacts quantified in this study are expected to contribute to complete risk assessments that would allow, e.g., listing *A. longifolia* as an European "official pest" (Council Directive 2000/29/EC) or prioritizing management of Conservation Areas invaded by *A. longifolia* in order to restore their natural communities before native species are excluded.

Box 7.3. Methodology (II): was it relevant to analyse diverse vegetation parameters?

Studies measuring impacts of invasive species and quantifying recovery after clearing frequently use parameters expressed by one single figure, *e.g.* species richness, plant cover, diversity indexes. These parameters are undeniably useful, and as such were also selected in this study. However, alone they may conceal relevant information. With this underlying notion a range of other parameters were selected (similarity indexes, turnover rates, plant traits and rank abundances curves) aiming to get more insights into the plant communities under comparison. Besides losing some information, some of the single figure parameters may lead to "misinterpretation" which would be clarified by analysing other parameters. For example:

1) similarity index revealed that invaded and non-invaded areas had very distinct species assemblages, with just a few species in common, and were in fact more different than suggested by species richness;

2) rank abundances revealed that some species contributing to richness of recently invaded areas were in fact very rare and therefore less likely to persist;

3) species contributing to similarities in species richness and cover through time were not always the same, as revealed by high turnover rates;

4) species traits allowed separation of species with distinct functions in the system (*e.g.,* exotic *vs.* native; ephemeral *vs.* perennial; wind dispersed *vs.* animal dispersed).

These results showed that over and above the advantages of not using single figure parameters, use a multiple range of parameters can also disentangle obscure patterns and trends.

WHY DOES *ACACIA LONGIFOLIA* HAVE SUCH LARGE IMPACTS IN PLANT DUNE COMMUNITIES?

Several characteristics of *A. longifolia* contribute to the changes observed after invasion, with environmental factors and ecosystem characteristics also contributing to some of the alterations.

1) *Acacia longifolia* is a prolific seed producer, the seeds are long-lived and despite many being lost before or after entering the seed bank vast numbers still accumulated in the sand dunes (chapter 3), frequently exceeding the numbers of seeds of native plant species (chapter 4). Areas invaded for

longer time have particularly large accumulations of seeds. The density of *A. longifolia* seedlings in the field (chapter 5), stimulated by clearing operations, were much lower than the density of seeds observed in the soil, probably due to the absence of fire which is an important stimulus for germination of this species (Pieterse & Cairns, 1986). This means that even after clearing a hidden legacy/threat remains in the soil assuring future invasions.

2) *Acacia longifolia* is a perennial species whose phyllodes decompose slowly (Marchante, 2008) resulting in the accumulation of a thick litter layer, which increases with time and is much thicker than that normally associated with uninvaded dune systems. This litter is richer in N than most of the native dune species, promoting changes in soil C and N cycle and fostering additional changes (Marchante *et al.*, 2008b) which persist after *A. longifolia* removal (Marchante *et al.*, 2009). The litter layer accumulated has major influence in the species that emerge and/or survive.

3) *Acacia longifolia*, both due to its different physiognomy (*i.e.*, a tree *vs.* the typical herbs, sub-shrubs and shrubs) and its high density in dunes, assures that soil coverage is much denser than that provided by native dune species, and especially so under the dense canopies of plants in long-invaded areas (chapter 2). This is partially explained by the faster growth rates (Peperkorn, Werner & Beyschlag, 2005) and higher ability to establish interactions with soil mutualists, mainly with symbiotic nitrogen-fixing bacteria (Rodríguez-Echeverría *et al*. 2009), which results in the competitive advantage of *A. longifolia*, particularly when the plants have survived through the seedling stage, (Werner *et al.*, 2008). Mortality of seedlings was high with few surviving (chapter 5). Its different physiognomy facilitated both the profound transformation of the landscape and the difficulty for other species to compete with it (chapter 2). Therefore, not only the invader matters, but its marked difference to the species of the recipient ecosystem also contributes to aggravate the level of impacts.

4) Being an exotic species, *A. longifolia* lacks its natural enemies that would normally curtail its uncontrolled growth.

5) Fire plays a fundamental role in the proliferation of the invasive stands through time by stimulating germination of *A. longifolia* seeds. This was particularly clear in the establishment of recently invaded stands that quickly proliferated after the summer fire of 1995, reaching more than 60% soil cover in less than eight years.

DO RESULTS SUGGEST THAT *TRICHILOGASTER ACACIAELONGIFOLIAE* SHOULD BE RELEASED IN PORTUGAL?

Tests performed in quarantine (chapter 6) and extensive experience in South Africa (Dennill, 1990; Dennill *et al.*, 1999) support the release of *T. acaciaelongifoliae* in Portugal (see Box. 7.4 for further steps before release). The overwhelming negative impacts of *A. longifolia* in dune communities (chapter 2; Marchante *et al.*, 2008b) showed the urgent need for management actions that reduce invader density and threat. The results of this study and South African experience show that the introduction of *T. acaciaelongifoliae* has great potential to help to achieve this aim. Recovery studies indicated that the invaded communities will respond positively to *A. longifolia* reduction with appearance of many native plant species though some exotics will also be present and cannot be ignored (chapter 5); this supports *T. acaciaelongifoliae* introduction. Studies of recovery potential, such as these, are scarce but are essential to anticipate the response of communities after the introduction of a biocontrol agent (Denslow & D'Antonio, 2005). There is no real risk of wasps laying eggs on plants other than *A. longifolia* (chapter 6) and any potential indirect non-target effects due to low levels of parasitoid acquisition (Hill & Hulley, 1995; Manongi & Hoffmann, 1995) should be minimal. Failing to establish due to changes of hemisphere or other environmental unsuitability is unlikely. Besides, any slight side effects will be more than offset by the substantial benefits that will accrue (both ecologically and economically) if the introduction of *T. acaciaelongifoliae* succeeds.

Box 7.4 Biocontrol further steps to release *Trichilogaster acaciaelongifoliae: brief guidelines**

1. Preparation of a pest risk analysis (PRA), because *T. acaciaelongifoliae* is likely to cause injury to a plant (*A. longifolia*).
2. Formal application to the Portuguese Authority (ICNB, Ministério do Ambiente e do Ordenamento do Território), including the PRA and asking for:
    a. Remove *T. acaciaelongifoliae* from the restrictions of Decreto-Lei n.º 565/99
    b. A permit to release *T. acaciaelongifoliae* in the wild
3. If permission to release is granted, establishment of contingency and monitoring plans.

PRA will include (EPPO, 2009):

Stage 1 - a brief Initiation – introductory section.

Stage 2 - a risk assessment with two major sections:

Section A. categorization of the “pest” (*i.e.,* biocontrol agent in this case) in the form of a binary decision tree

Section B. assessment of the probability of spread and potential economic consequences (performed only if the Section A concludes that the organism can be a pest).

Stage 3 – a Pest Risk Management to determine if the risk is acceptable and to identify management options.

Before or during stage 3, a public consultation should take place.

* based on framework followed in UK, which is likely to have created precedents; more details in chapter 6.

The use of *T. acaciaelongifoliae* should be integrated into a larger management program, *e.g.* including other control strategies, aimed at restoring the whole system, rather than being developed separately (Denslow & D'Antonio, 2005). Before release, realistic integrative goals should be set to subsequently evaluate the level of success of the project. Such goals should include both responses of the community and behaviour of the agent, e.g.: 1) successful establishment of the wasp; 2) reduction of *A. longifolia* cover to non problematic levels; 3) major decrease of seed production interrupting the continuous seed bank enrichment; 4) recovery/increase of native species diversity, and, consequently, 5) recover of ecosystem services. Baseline assessments, though scarce, are essential to compare with future post-release data and quantify the effectiveness of the biocontrol agent (Harley & Forno, 1992). In this thesis several of these assessments were made (quantification of annual seed production and resulting seed bank accumulation (chapter 3), *A. longifolia* and native species cover (chapter 2), responses of the vegetation to *A. longifolia* removal (chapter 5)) and will be valuable for future management. Growth phenology has also been studied for Portugal (Morais *et al*., *unpublished data*) and will be similarly useful.

As suggested above, when considering invasive plant management (Holden, Fowler & Schroeder, 1992; Murphy & Evans, 2009; Pyšek & Richardson, 2010; Wilson *et al.*, submitted), biological control might be the only sustainable, cost effective and environmentally-sound way to prevent the escalation irreversible damages that *A. longifolia* will inevitably inflict on the ecology and biodiversity of whole communities of native organisms in Portugal, and further afield in Europe.

CONCLUSIONS

1. Management should not be delayed. Impacts of *A. longifolia* escalate with time and dispersal patterns indicate that stands will keep advancing so delaying management will aggravate the problem. Management actions should start before the resilience of the system is too low. In areas invaded for long time “doing nothing” may sometimes be the best option, *i.e.*, when restoration is predicted to be very hard/impossible to achieve. This does not to seem to be the case for the invasion by *A. longifolia* in SJNRD, yet, as many native species were observed slowly (re)colonizing. However, if invaded areas are left, managers need to be aware that more vigorous and probably more expensive management efforts will then be needed if the invaded system is to be restored in the future.

2. Areas and species need to be prioritized:

2.1. New foci of *A. longifolia* and/ or other exotic species should be targeted for rapid response, *i.e.*, local eradication, control or containment depending on the situation. Seeds of *A. longifolia* were found several meters away from the invaded stands and new invasion foci occur hundreds of meters from the main thickets (chapter 3). These foci are manageable pockets of new invasion and should be prioritised for removal (Rejmánek & Pitcairn, 2002). The new foci of other invasive plants detected after clearing, *e.g. Cortaderia selloana*, *C. edulis* and *Conyza* spp. (chapter 5), should be managed first, or at the same time as *A. longifolia*, to prevent them becoming a bigger problem after *A. longifolia* removal.

2.2. Recently invaded areas have higher prospects of successful recovery (chapter 5) and so should also be prioritized for control actions.

3. Follow-up control is crucial:

3.1. Reinvasion by *A. longifolia* and secondary invasion by other exotic species occurred after removal of the invader (chapters 4 and 5). This threat intensifies as the invasion progresses because *A. longifolia* seed banks increase, even though many seeds are lost and the remaining loose some viability with time and become increasingly more dependent on a stimulus to germinate. *Acacia longifolia* saplings in sand dunes can be hand pulled easily when they reach 20 cm – 40 cm, avoiding waste of resources and time to eliminate smaller seedlings that will not survive anyway. Secondary invasion by *C. edulis*, particularly in recently invaded areas, *C. selloana* and *Conyza* spp. was also observed and cannot be ignored.

3.2. *Acacia longifolia* seeds were viable for at least six years (chapter 3), but are expected to remain viable for even more years (Cavanagh, 1980; Daws *et al.*, 2007). As the time to first reproduction is about two years, or even less for plants originating from resprouts, after removal of the invader the cleared area should be monitored, at least annually for six years, to set follow-up (and latter, maintenance) control actions in place (Grice, 2009). Moderate fire can be used to deplete the seed bank (Galatowitsch & Richardson, 2005) and will reduce the duration of follow up control.

4. Non-invaded areas need also to be monitored. Although there was no *A. longifolia* trees in these areas, seeds were found in non-invaded areas and may initiate invasions if stimulated by disturbance (*e.g.,* fire).

5. Removal of thick litter layers may be beneficial for recovery. Removing the thick litter layers (of long-invaded areas) eliminated many seeds of *A. longifolia* and facilitated the recovery of the areas. Removal of thinner litter layers (recently invaded areas) led to loss of species in the seed bank (chapter 4) and in the field (in 2002, chapter 5), but migrating species propagules compensated for that loss. The effort required to remove litter was not very cost-effective, particularly with thinner litter layers; moderate prescribed fire can be used to eliminate the thick litter layer and simultaneously destroy many seeds of the invasive species (Richardson & Kluge, 2008).

6. Additional restoration measures may be needed to restore native plant communities, particularly in the areas invaded for a long time. Although many native species (re) colonised invaded areas after clearing, including several species characteristic of the dunes, many others were missing and other exotic species were present. This needs to be considered when planning recovery strategies. Autogenic recovery does not seem to be sufficient to allow full system recovery after clearing but will make a significant contribution (chapter 5). Transplanting of native species should be considered (Galatowitsch & Richardson, 2005), unless a novel or hybrid ecosystem, with a species assemblage distinct from the historic mixture, is set as a target instead (Hobbs *et al.*, 2006).

Box 7.5 Could different management options in the past have solved the erosion problem without introducing *A. longifolia*?

It can be argued that without the introduction of the invasive *A. longifolia* (and other exotics such as *C. edulis*) sand from dunes would have been lost due to erosion, *i.e.*, "sand dunes with *A. longifolia*" are better than "no sand dunes at all". An alternative could have been to use engineering solutions to stabilise sand, but these are in general very expensive.

Marram grass (*Ammophila arenaria*), a perennial herb typical from Portuguese foredunes, has been used successfully to stabilize sands, both in the native range (Schreck Reis, Antunes do Carmo & Freitas, 2008) and in exotic ranges (*e.g.,* south-east Australia (Heyligers, 1985) and California (Buell, Pickart & Stuart, 1995)). In the exotic range, it has become invasive, probably due to reduction in the abundance of root feeding nematodes, amongst other factors (van der Putten *et al.*, 2005). In its native range, even with its growth, longevity and abundance reduced due to natural enemies, marram grass is very effective in stabilizing sands and could have been used more proactively.

7. Biocontrol is a promising and sustainable option to integrate in a complete management program. Recovery studies showed that the system will react well to *A. longifolia* removal even though some other exotics will benefit from the reduction and have to be considered. In this context, and considering the results of specificity testing the introduction of the studied biocontrol agent – *T. acaciaelongifoliae* – is recommended as it will most likely be the most sustainable option to reduce *A. longifolia* to non-problematic levels. A combination of biological control together with initial control by mechanical methods will probably be the best option.

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