UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS

Factors involved in natural regeneration of *Eucalyptus globulus*

Documento Definitivo

Doutoramento em Biologia
Especialidade em Ecologia

Andreia Sofia Gonçalves dos Anjos

Tese orientada por:
Professora Doutora Cristina Máguas
Professor Doutor Sergio Chozas

Documento especialmente elaborado para a obtenção do grau de doutor

2024

UNIVERSIDADE DE LISBOA

FACULDADE DE CIÊNCIAS

Factors involved in natural regeneration of *Eucalyptus globulus*

Doutoramento em Biologia
Especialidade em Ecologia

Andreia Sofia Gonçalves dos Anjos

Tese orientada por:
Professora Doutora Cristina Máguas
Professor Doutor Sergio Chozas

Júri:

Presidente:

- Cristina Maria Branquinho Fernandes, Professora Catedrática e Presidente do Departamento de Biologia Vegetal da Faculdade de Ciências da Universidade de Lisboa

Vogais:

- Doutor Joaquim Manuel Sande da Silva, Professor Coordenador Principal da Escola Superior Agrária de Coimbra do Instituto Politécnico de Coimbra;
- Doutor Paulo Alexandre Martins Fernandes, Professor Associado do Departamento de Ciências Florestais e Arquitetura Paisagística da Universidade de Trás-os-Montes e Alto Douro;
- Doutora Catarina Isabel Antunes Gonçalves, Técnica Superior de Investigação da RAIZ – Instituto de Investigação da Floresta e Papel, na qualidade de individualidade de reconhecida competência na área científica;
- Doutor José Miguel Oliveira Cardoso Pereira, Professor Catedrático do Instituto Superior de Agronomia da Universidade de Lisboa;
- Doutora Cristina Maria Filipe Máguas da Silva Hanson, Professora Associada com Agregação da Faculdade de Ciências da Universidade de Lisboa (Orientadora).

Documento especialmente elaborado para a obtenção do grau de doutor
Em colaboração com o Instituto de Investigação da Floresta e do Papel (RAIZ)

2024

This study was funded by FCiências.ID and RAIZ – Forest and Paper Research Institute in the frame of Andreia Anjos PhD scholarship, as well as by FCT and CE3C - Center for Ecology, Evolution and Environmental Changes, Research Unit grant number UIDB/00329/2020.

Nota Prévia

A presente tese apresenta resultados de trabalhos já publicados ou em preparação para publicação (capítulos 2 a 5), de acordo com o previsto no nº 2 do artigo 25º do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicado no Diário da República II série nº 57 de 23 de março de 2015. Tendo os trabalhos sido realizados em colaboração, o candidato esclarece que participou integralmente na conceção dos trabalhos, obtenção dos dados, análise e discussão dos resultados, bem como na redação dos manuscritos.

Lisboa, Julho de 2024

Andreia Sofia Gonçalves dos Anjos

Agradecimentos

À Professora Cristina Máguas e ao Professor Sergio por terem atravessado cada capítulo desta tese comigo; à Professora Otília por ter embarcado nesta aventura do eucalipto e por ter deixado sempre a sua palavra sábia e atenta

A Sergio por la paciencia mientras yo era una novata y no sabía ni abrir la tabla en el R; por el descubrimiento del mundo de las publicaciones y de las respuestas a los revisores; y por convertirte en el mensaje mañanero “¿café?”, siempre con aquella buena disposición, palabras sabias, leves y relajadas

Ao RAIZ: Cristina Marques, Nuno Borralho e Carlos Valente, pelo acompanhamento constante e por todas as palavras reconfortantes nas diferentes etapas de cada capítulo; ao José Alexandre por toda a simpatia e disponibilidade, paciência em ensinar a fenologia do eucalipto e pelos emails a dar notícia da meteorologia; ao Filipe Sousa, Rui Gomes e Sr. Leonel por me mostrarem as plantações que há por aí fora e por me transmitirem o conhecimento da gestão florestal

À minha mãe – esta tese também é tua. Por todo o apoio, compreensão, ajuda, pela preocupação nas imensas viagens que fiz para acabar este doutoramento; aos meus avós (que embora já não estejam cá) sempre lhes senti a energia e o orgulho por irem ter uma neta Doutora; aos meus padrinhos e primos pelos brindes e momentos bem passados; ao J Luz pela constante preocupação com o meu bem-estar e felicidade

À Joana, a que começou como colega de campo sem nos conhecermos de lado de nenhum e que se tornou a companheira de vida. Pelo ombro, pelo abraço e pelo conforto, mas também pela celebração de todas as pequeninas vitórias que tornaram esta tese um PDF. Por teres amostrado, recolhido, conduzido, lido, relido, revisto, por teres feito parte desta tese desde o início e sem ti não seria a mesma coisa; e à família Jesus e ao João por me terem acolhido

À Catz e aos Beberetes, Jacinto e Estorninho, por compreenderem cada peripécia como se fosse na primeira pessoa e por serem companhia, amparo e gargalhadas

À Adriana, Inês e Teresa por não ser preciso falar porque me entendem a léguas e por darem sempre aquela motivação extra e energia boa

À Andreia Silva, Inês Gonçalves, Joana Castro e à família que, entretanto, foi crescendo, por nos mantermos desde a licenciatura e por nos entendermos como se fosse diário

À Adelaide, Carla, Cris, Graça e ao restante ESFE por tanto ouvirem sobre eucalipto, por verem a confusão dos meus gráficos, por ajudarem a decidir a palete de cores e o tamanho das letras e por me fazerem sentir parte do grupo

Àquelas que me acompanharam nos quilómetros e quilómetros e quilómetros por este país fora: Bárbara Charlotte, Carla Saraiva, Catz, Deni, Inês Mirra, Joana Escuteira e Mariana Estorninho

Ao Rogério (Doutor, vou ser Doutora!) por todas as horas e horas e horas que me ouviu a falar, mas também por vibrar com as pequenas e grandes vitórias que fui celebrando

Migos, o QUASE já era – desta vez já está! Obrigada a todos por fazerem parte da minha vida!

[A vocês que estão aí em cima a olhar por mim – à nossa!]

Àqueles a quem o fogo tudo tirou e
a quem a terra quase tudo dava.
Àqueles que se rodeiam de natureza e para quem os 242000 hectares eram casa.
Àqueles a quem o fogo trouxe solidão e para quem a perda foi avassaladora.
Àqueles que se conhecem pelos géneros e para os quais todos somos vizinhos.

Àqueles que reergueram a terra e ainda estão a recuperar a herança.
Àqueles que ainda assim estão seguros e enraizados à vida de sempre.
Àqueles para quem 2017 ainda é pânico, dor e perda - a quem agora conto uma história do ponto de vista arbóreo.
Àqueles cuja morada pertence à natureza, como o eucalipto aqui morada tem.
Àqueles que recordam, resistem, persistem.
- Juca. -

[Porque para nós foi trabalho e estudo – mas para muitos fica a memória do que se perdeu]

Abstract

Identifying factors that allow an introduced species to reproduce and spread is critical, particularly for high-value species, such as *Eucalyptus globulus*, one of the most planted hardwood trees worldwide. In Portugal, this Australian species covers 26% of the forested area in managed and unmanaged plantations and as old, isolated trees (called seed-trees). While its dispersal behaviour is known, new scenarios are emerging: altered fire regimes and tree breeding programs. Thus, this thesis addresses i) the impact of off-season fires, pre-fire management and the presence of seed-trees along with ii) local factors that influence *E. globulus* establishment; iii) the phenological behaviour and reproductive structures production and iv) the capsule morphology, seed set and germination rate of improved trees and seed-trees, to ultimately assess this species dispersal risk. To achieve that, managed and unmanaged *E. globulus* plantations and areas adjacent to seed-trees were sampled for sapling cover and biotic and abiotic factors in unburnt and burnt areas affected by the spring and autumn fires. Also, phenological behaviour, reproductive structures' production and seed germination were studied on clonal stands and compared with seedling-based stands and old seed-trees, in mesic and xeric sites. Our findings reveal that i) sapling establishment is limited due to pre-fire management; ii) natural regeneration increases after autumn fires; iii) seed-trees constitute a source of propagules; iv) saplings are sensitive to post-fire conditions; v) tree-related traits influence sapling cover in plantations, whereas seed-trees buffer unfavourable conditions; v) clonal trees exhibit shorter flowering, fewer reproductive structures, and higher phenological variability and behave differently among each other; vi) clonal seeds display a high germination rate and vii) seed-trees produce fewer seeds while maintaining their germination rate over time. This information aims to draw attention to *E. globulus* improved plantations and the presence of seed-trees in the landscape management strategies.

Keywords: Exotic species; Off-season fires; Environmental factors; Tree improvement

Resumo alargado

As florestas plantadas são fundamentais para a economia global, impulsionando o crescimento socioeconómico ao responder à crescente procura por produtos lenhosos e outros serviços essenciais. Contudo, a utilização de espécies exóticas como resposta a esta procura pode ter um impacto negativo no solo, no ciclo da água, na paisagem e na biodiversidade, comprometendo assim o equilíbrio dos ecossistemas. Adicionalmente, a possível expansão além das áreas plantadas, pode resultar em processos de invasão que comprometem a sobrevivência e a permanência das espécies nativas. Deste modo, é de extrema relevância estudar os fatores que afetam a regeneração natural de espécies exóticas em zonas não nativas. Devido à sua importância para a indústria da pasta do papel, *Eucalyptus globulus* é uma das espécies de árvore mais plantada em todo o mundo. É a segunda espécie exótica com maior área ocupada na Europa, abrangendo aproximadamente de 1.46 milhões ha, especialmente na Península Ibérica. Em Portugal, ocupa um quarto da área florestal do país (aproximadamente 845 000 ha), tanto em plantações com gestão intensiva (com corte de árvores e limpezas frequentes do subcoberto), como em plantações não geridas ou abandonadas, e ainda árvores isoladas e velhas, denominadas como sementões, muitas vezes utilizadas para delimitação de plantações e/ou propriedades. Embora o potencial invasor desta espécie tenha sido amplamente estudado, novos cenários, como a alteração do regime de fogos e o desenvolvimento de programas de melhoramento de árvores podem alterar a regeneração natural desta espécie. Com o aquecimento global e a correspondente diminuição da precipitação, verifica-se um alargamento da época de fogos além dos meses de Verão, que pode alterar a dinâmica e a regeneração pós-fogo desta espécie tão adaptada a estes regimes. Por outro lado, a sua elevada importância económica promoveu o desenvolvimento de programas de melhoramento a fim de corresponder à elevada procura, através da seleção de material mais produtivo e com maior qualidade. Neste sentido, pretendeu-se avaliar de que forma *E. globulus* está a responder a estes cenários e quais os fatores que influenciam a sua regeneração natural nestes novos contextos. Assim, este estudo i) avaliou o impacto dos fogos fora de época, da gestão pré-fogo e da presença de sementões na dispersão do eucalipto; ii) identificou os fatores locais que influenciam o seu estabelecimento após-fogo; iii) acompanhou o comportamento fenológico e a produção de estruturas reprodutoras; iv) caracterizou as cápsulas, a produção de sementes e a taxa de germinação de árvores melhoradas. Esta tese é composta por seis capítulos, incluindo uma introdução geral ao tema e quatro capítulos correspondentes a artigos científicos publicados (2º ao 4º capítulos) ou em

preparação para submissão (5º capítulo) em revistas internacionais de arbitragem científica indexadas no *Science Citation Index*. Por último, são discutidos os resultados finais, apresentadas as conclusões gerais e sugeridas recomendações para uma gestão mais sustentável desta espécie florestal, focando principalmente na sua dispersão e potencial capacidade invasora.

No **Capítulo 1** é descrita a problemática relativa à introdução de espécies em florestas plantadas, destacando *E. globulus* e o seu potencial invasor, bem como as características gerais, comportamento fenológico e fatores que afetam o seu estabelecimento. Também são abordadas a problemática dos fogos fora de época e a plantação de árvores melhoradas, demonstrando a necessidade de esclarecer qual o comportamento desta espécie nestes novos cenários. Assim, e apesar da elevada adaptação e regeneração de *E. globulus* após a ocorrência de um fogo sejam conhecidas, no **Capítulo 2** avaliou-se de que forma os fogos fora de época, a gestão pré-fogo e a presença de sementões afetam a dinâmica pós-fogo desta espécie exótica. Deste modo, a dispersão de *E. globulus* foi estudada um ano após os grandes fogos de junho e outubro de 2017 (ocorridos antes e após a época oficial de fogos em Portugal), em plantações geridas e não geridas, bem como em áreas geridas e não geridas adjacentes a sementões. Após os fogos de Outono, observou-se maior regeneração natural, principalmente em áreas não geridas, uma vez que a gestão pré-fogo restringe o estabelecimento de plântulas. Verificou-se ainda que os sementões são uma fonte de propágulos resultando também em eventos de dispersão a maiores distâncias da árvore-mãe. Ainda neste contexto, estimou-se a importância relativa dos fatores locais na regeneração pós-fogo (**Capítulo 3**). O estabelecimento de plântulas é significativamente influenciado por fatores bióticos e abióticos; quando resultantes de plantações, as plântulas são afetadas por variáveis relacionadas com as árvores-mãe (idade e altura), enquanto a presença de sementões mitiga as condições adversas do meio ambiente, promovendo o estabelecimento. Por outro lado, no **Capítulo 4**, foi estudado o padrão fenológico e a produção de estruturas reprodutoras em três plantações clonais (genótipos melhorados), comparando com uma plantação seminal (não melhorada), em dois locais climaticamente distintos. Foram acompanhados dois períodos de floração consecutivos, de modo a avaliar o efeito do melhoramento genético no comportamento reprodutor de árvores melhoradas. Em comparação com a plantação seminal, as três plantações clonais apresentaram um período de floração mais curto, uma menor produção de estruturas reprodutoras e um comportamento mais variável das árvores clonais entre os locais. Foi também registada uma elevada variabilidade entre os clones, incluindo um clone que não produziu estruturas reprodutoras, um clone que apresentou uma floração mais prolongada e abundância de estruturas reprodutoras semelhante ao material seminal em condições mais quentes, e um clone com variabilidade interanual, apresentando menor capacidade reprodutora do que as árvores seminais e melhor desempenho em condições mais húmidas. Além disso, no **Capítulo 5**, foram estudadas características referentes à biologia reprodutiva de árvores clonais e de sementões. Durante um ano foram recolhidas cápsulas de árvores em plantações clonais e seminais, bem como de sementões, em dois locais climaticamente distintos de Portugal (Coimbra, mais húmido e fresco, e Pegões, mais seco e quente). As cápsulas recolhidas foram caracterizadas relativamente ao seu ano de produção (2018 a 2021), tamanho, peso e número de sementes e posteriormente as sementes foram colocadas a germinar. O tamanho e peso das cápsulas são influenciados pela proveniência das árvores que lhes deram origem, bem como pelas condições climáticas. As árvores clonais produzem cápsulas com número e taxa de germinação de sementes semelhante às árvores seminais, enquanto os sementões possuem um menor número de sementes por cápsula, mas apresentam uma capacidade germinativa superior, mesmo em sementes mais velhas. Por fim, no **Capítulo 6**, discutem-se os principais resultados de cada capítulo e referem-se as conclusões gerais da tese. Além disso, com base nos resultados deste estudo são apresentadas recomendações para uma gestão mais eficaz das áreas plantadas relativamente ao controlo de potenciais eventos de recrutamento e dispersão.

Em resumo, os resultados deste trabalho põem em evidência que, embora a ocorrência de fogos seja indispensável para a dispersão de *E. globulus,* a data do fogo, associada às condições climáticas posteriores, é determinante para a ocorrência de eventos relevantes de expansão, uma vez que as plântulas são muito sensíveis às condições pós-fogo. Por outro lado, verificou-se que a gestão pré-fogo restringe o estabelecimento de plântulas, essencial para o controlo da expansão. Aliada à gestão intensiva, i.e., corte de árvores em ciclos frequentes impedindo a maior produção de cápsulas, a seleção de árvores com reduzida ou nenhuma capacidade reprodutora, elimina assim qualquer eventual dispersão, mesmo com a ocorrência de fogos frequentes ou o abandono de áreas plantadas. Além disso, a escolha dos clones a implementar deverá ter em conta o local da plantação, considerando assim o comportamento de cada genótipo. No entanto, especial atenção deverá ser dada à existência de árvores isoladas (sementões), uma vez que são uma fonte adicional de propágulos muito relevante para a dispersão. De facto, após os fogos, estas árvores rebentam ao longo do tronco

e, além da grande capacidade reprodutora e sucesso germinativo, promovem condições favoráveis ao estabelecimento de novas plântulas.

Esta tese também clarifica a resposta de *E. globulus* às alterações climáticas e às estratégias que têm vindo a ser desenvolvidas focadas na maior produtividade, salientando as limitações associadas à ecologia da espécie, alertando para a potencial naturalização em Portugal. Contudo, é de salientar a necessidade de uma monitorização frequente, principalmente devido à elevada e crescente área plantada e às alterações das condições ambientais.

Palavras chave: Espécies exóticas; Fogos fora de época; Fatores ambientais; Melhoramento genético

Table of Contents

Chapter 1

GENERAL INTRODUCTION, AIMS AND THESIS OUTLINE

General Introduction

Human activity significantly impacts biodiversity and ecosystems (Steffen *et al*. 2007). Global change imposes many threats to the habitats and species, from which land-use transformation and overexploitation outstand (Tilman *et al*. 2017). In particular, the growing demand for forestry products causes land homogenization, threatening biological diversity and ecosystem functioning, despite providing significant economic and social benefits (Liu *et al*. 2018). Fast-growing species, such as the tree genus *Eucalyptus*, one of the most widely planted hardwood trees worldwide, are attractive due to the rapid economic return (Messier *et al*. 2021). *Eucalyptus globulus* is a globally introduced planted species due to its fast growth and superior wood properties (Potts *et al*. 2004), raising concerns about its direct impacts on new environments and its risk of dispersal beyond planted areas, particularly after a fire when natural regeneration is highly potentiated (Águas *et al*. 2014). However, climate change alters fire regimes, extending the fire season outside the summer months (Moriondo *et al*. 2006). These off-season fires are becoming more common and may alter *E. globulus* post-fire behaviour, improving recruitment events and dispersal. As a result, more sustainable forest management is required to meet the growing demand for forestry products while reducing planted areas and the risk of expansion. Additionally, genetic tree improvement programs have emerged, and clonal plantations are been implemented to increase wood yield and quality while decreasing planted areas.

Moreover, the characteristics that make *E. globulus* attractive for the pulpwood industry, such as rapid growth, high fecundity, and tolerance to a wide range of climatic and soil conditions also make it potentially invasive. As a result, identifying factors that promote this species' recruitment and establishment in this context is critical. Furthermore, a better understanding of the behaviour of this species outside of its native range will aid in the management of sustainable forests.

1.1 Forest plantations and their impacts

Over the last two centuries, the world's population has grown exponentially, and the high demand for products and shelter is transforming natural areas into forestry, agriculture, farmlands, and urban areas (Millennium Ecosystem Assessment 2005). These land-use changes result in habitat fragmentation (Miller and McGill 2017), soil and water cycles alteration (Wills *et al*. 2017; Levia *et al*. 2020), biodiversity losses (Giam *et al*. 2010), and invasive species dispersal (Lonsdale 1999; Richardson and Rejmánek 2011) leading to environmental degradation (Steffen *et al*. 2007) and endangering the entire planet. One of the most striking land-use changes is the establishment of forest plantations, which are generally monocultures of one or two species and are intensively managed for economic purposes such as timber, fibre, energy, and non-wood products (FAO 2020).

Planted forests, as opposed to native forest areas, have expanded from approximately 170 million to 293 million hectares (ha) in the last thirty years, accounting for approximately 7% of the global forested area. There are 131 million ha monocultures among these, with 44% occupied by introduced species (FAO 2020) that are being moved to non-native ranges due to the high quality of the products (Payn *et al*. 2015), resulting in significant economic and social benefits for many countries (Dyck 2003). Nevertheless, there are concerns about the ecological risk and negative effects of introduced species on ecosystem functioning, such as decreased biodiversity (Vilà *et al*. 2011), landscape fragmentation (Carnus *et al*. 2006), altered water and soil cycles (Liao *et al*. 2012; Thompson *et al*. 2014), and changes in fire hazard (Calviño-Cancela *et al*. 2016). In addition to these threats to local biodiversity, introduced species may be able to establish and reproduce in the novel environment, allowing them to spread outside of plantation areas compromising the integrity of native communities (Essl *et al*. 2010).

1.2 Invasion risk of plantation forests

Plant introduction is one of the first stages of the biological invasion process, wherein exotic species are introduced into a new ecosystem by humans, whether deliberately or inadvertently. Such introductions are related to several purposes, including horticulture, food, and agroforestry, with approximately 13% used as plantation forestry (Richardson and Rejmánek 2011). The impact of these introduced species, mostly planted on a large scale as intensively managed monocultures is currently regarded as one of the greatest threats to global diversity (Brundu *et al*. 2020).

After an introduction, individuals may occasionally reproduce outside the introduction area, but they do not form self-sustaining populations and are thus classified as casual species (Lambdon *et al*. 2008). However, exotic species may overcome various biotic and abiotic barriers due to favourable soil and climate conditions, competitive superiority, and/or habitat disturbance. As a result, these species can reproduce and survive, self-sustaining the population without further human intervention, allowing them to become naturalized

(Blackburn *et al*. 2011; Dodet and Collet 2012). The critical point is reached when some of these naturalized species can produce offspring on a large scale and spread to great distances from the mother plant (as mentioned by Richardson *et al*. (2000), more than 100 m in less than 50 years), being classified as an invasive species (Blackburn *et al*. 2011). These authors proposed a unified framework for populations at various stages of the invasion process (Fig. 1.1).

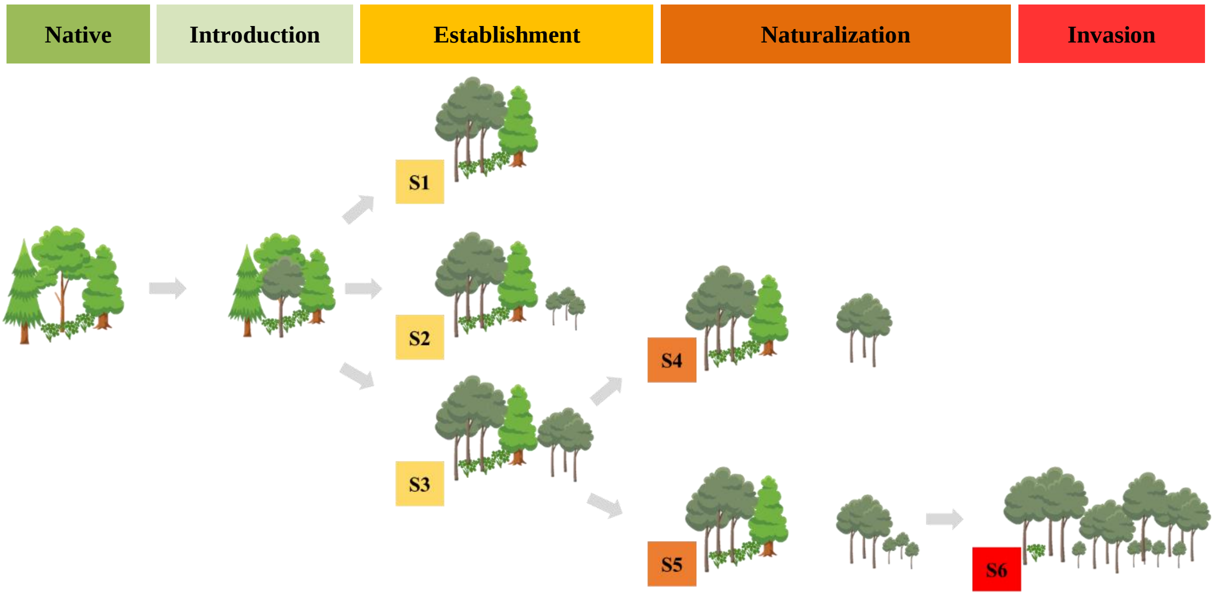


Figure 1.1 Diagram of the stages involved in the invasion process modified after Blackburn *et al*. (2011): **S1** - Individuals that are surviving in a new environment but are unable to reproduce; **S2** - Individuals surviving in a new environment, capable of reproducing but unable to self-sustaining a population; **S3** - Individuals capable of reproducing and self-sustaining in a new environment; **S4** - Individuals surviving in a greater distance from the introduction area, resulting in a self-sustaining population in a new environment; **S5** - Self-sustaining population in a new environment, with individuals surviving and reproducing in a greater distance from the introduction area; **S6** - Individuals dispersed across multiple locations, surviving and reproducing, reaching the status of invasive species.

Propagule pressure accounting for residence time, i.e., the time since introduction, is critical for the success of the introduction-naturalization-invasion continuum. Naturally, plantation density has an impact on invasion dynamics. For example, a higher number of individuals arriving in the new environment (propagule pressure) can facilitate species dispersal (Lockwood *et al*. 2005; Pyšek *et al*. 2009; Simberloff 2009); also, a greater number of individuals producing offspring for longer periods (residence time), will increase the odds of a species to be able to establish, spread, and invade new environments (Pyšek and Jarošik 2005; Rejmánek *et al*. 2005). However, introduced species must overcome various barriers to move through the various stages of the invasion process. Williamson (2006) mentioned several variables that affect each barrier: economic factors influence the introduction, while ecological and geographic factors affect individual dispersal. Furthermore, characteristics related to species reproduction, specifically seed ecology, are critical for a potential invasion (Dawson *et al*. 2009).

Nevertheless, the resistance of the new environment to colonization (invasibility) also plays a role in the invasion process and is highly dependent on disturbance (Lonsdale 1999; Lake and Leishman 2004). In this sense, a low propagule pressure in an environment with less capacity to resist, such as a highly disturbed area, can result in an invasion. In the case of forestry species introduced in favourable environments for development, their fast growth, high productivity and tolerance to a wide range of climatic conditions, reproductive precocity and large seed production (Turnbull 1999; Richardson and Rejmánek 2011) contribute to the success of the species and trigger invasion events (Essl *et al*. 2010). The lack of pests and interspecific competition in the introduced range also potentiates the invasion process (Nair 2001; Golivets and Wallin 2018).

Therefore, sustainable management of exotic planted trees, focusing on the natural regeneration dynamics and expansion risk, is extremely relevant (Brundu *et al*. 2020).

1.3 *Eucalyptus globulus* Labill. plantations

One of the most widely planted hardwood tree genera is *Eucalyptus*. Due to their high growth rates, wide adaptability to soil and climate conditions, low incidence of disease and high resistance to pests, ease of management through coppicing, and valuable wood and non-wood products (Turnbull 1999; Booth 2013) there are currently more than 100 countries across six continents planting these trees (Iglesias Trabado and Wilstermann 2009), in a variety of environments ranging from tropical, subtropical, and temperate forests (Byrne 2008).

Despite the socioeconomic benefits, there is criticism of the replacement of native forests with eucalypt plantations due to potential ecological impacts such as soil properties' alteration (Mallen-Cooper *et al*. 2022) through heavy nutrient and water consumption that lead to soil erosion (Daba 2016), ecosystem functioning changes (Ferreira *et al*. 2019), and biodiversity loss (Goded *et al*. 2019; Castro *et al*. 2022). Moreover, there is a great controversy regarding its invasive potential due to the vast planted area and high seed production (Tomé *et al.* 2021), with some species classified as invaders (Henderson 2001; Forsyth *et al.* 2004; Rejmánek and Richardson 2013; Badalamenti *et al*. 2018). Furthermore, despite mature eucalypt trees' high seed production (more than 4.000 seeds/m^2) (Rejmánek and Richardson 2011), previous studies revealed low levels of seedling establishment (Callaham *et al*. 2013; Lorentz and Minogue 2015; da Silva *et al*. 2016). In fact, eucalypt establishment in new environments was hampered by limited seed dispersal, high seedling mortality, and a lack of ectomycorrhizal fungi (Rejmánek and Richardson 2011).

Although this genus has approximately 750 species, only nine are economically significant, accounting for 90% of the current eucalypt plantation (Stanturf *et al*. 2013). One of the most commonly cultivated *Eucalyptus* species is *E. globulus* Labill. (Tasmanian blue gum), an evergreen, broadleaf tree described by Labillardiére in 1799 (Eldridge 1993). It belongs to the Myrtaceae family and is native to Tasmania, the Bass Strait Islands, and southeast Australia (Kirkpatrick 1975). It is genetically variable across its geographic range, with 13 geographical races and 20 subraces described (Dutkowski and Potts 1999).

Eucalyptus globulus was the first species of the genus *Eucalyptus* to be widely planted in exotic ranges due to its use in the paper industry (Jacobs 1979), and it is now the second exotic tree with the largest area in Europe, ca. 1.46 million ha (Brus *et al*. 2019), mostly in the Iberian Peninsula (Cerasoli *et al*. 2016). This species was introduced in Portugal in the middle of the 19th century, with the first record in 1829 (Goes 1977; Potts *et al*. 2004; Alves *et al*. 2007). However, the first plantations were established in 1870 for railway material and were not used as a source for the pulp industry until the 1940s (Jacobs 1979; Alves *et al*. 2007). Since the 1950s, the planted area has increased greatly (Nunes *et al*. 2019) and currently occupies about 26% of the forested area of the country (ca. 845 000 ha) (ICNF 2019), with great economic return (Nunes *et al*. 2019).

The large majority of the area occupied by this exotic species was intentionally planted, and approximately one-third is well managed by the industrial pulp and paper companies (Feliciano *et al*. 2015), typically in short-rotation systems, coppiced every 10 to 12 years along three rotations, and self-thinning of shoots to optimize biomass production (Jacobs 1979; Alves *et al*. 2007) (Fig. 1.2a). In addition to tree-focused actions, intensive management practices include periodic understory vegetation removal and soil preparation for replanting, resulting in a simplified vegetation structure and diversity (Vaz *et al*. 2019). This approach impacts soil quality but decreases fire risk by removing available fuel (Catry *et al*. 2013; Benali *et al*. 2021). Furthermore, seed accumulation in the canopies is reduced due to short rotation cycles, resulting in a low potential for establishment outside plantation areas (Fernandes *et al*. 2016a; Queirós *et al*. 2020). Under managed regimes, trees are cut before they reach their maximum reproductive capacity, which takes about seven to ten years (Kirkpatrick 1975; Eldrige *et al*. 1993). Moreover, machinery movements and regular clearing actions naturally damage or kill potential seedlings, aiding in offspring establishment control.

Nonetheless, while propagule pressure is reduced in managed conditions, due to a short residence time (Gordon *et al*. 2012; Larcombe *et al*. 2013), propagule pressure increases in unmanaged and unharvested situations (Fig. 1.2b) due to the presence of older trees that reach high levels of seed production, potentially enhancing seedling establishment (Águas *et al*. 2017a; Deus *et al*. 2019; Queirós *et al*. 2020). In addition, some plantations are abandoned, easing the transition to mixed communities (Larcombe *et al*. 2013), altering landscape dynamics and allowing fuel accumulation and increasing fire spread (Fernandes *et al*. 2011).

Furthermore, as a result of this abandonment, and also because they are frequently used to delimit plantations and properties (pers. obs.), there are scattered in the landscape old uncut *E. globulus* isolated trees (Fig. 1.2c) with high canopies that could be a huge propagule source due to a major reproductive output. However, to our knowledge, these isolated trees (also known as seed-trees) were not identified as a driver for *E. globulus* expansion.

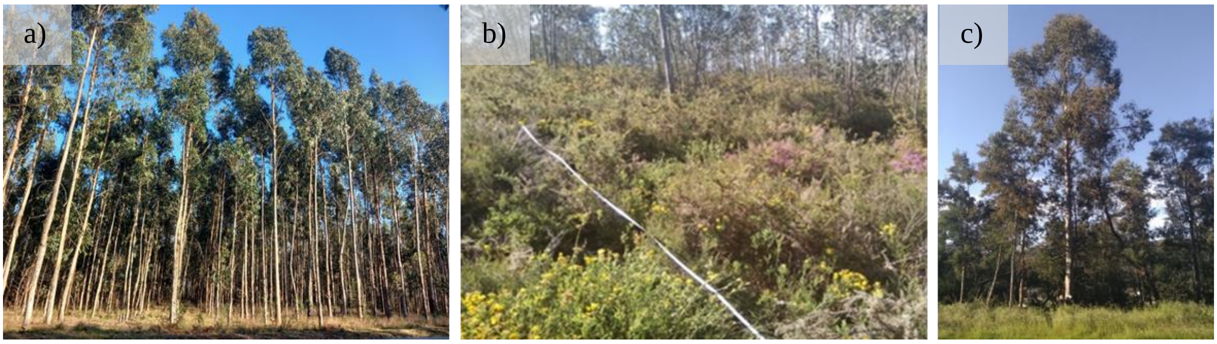


Figure 1.2 Managed (a) and unmanaged (b) *E. globulus* plantations and an isolated seed-tree (c).

Similar to other species in this genus, *E. globulus* invasive behaviour is controversial: some studies consider this species as an invader or with high risk in Europe, the Americas, Egypt, New Zealand and the Pacific and the Indian Ocean islands (Sanz-Elorza *et al*. 2001; Gassó *et al*. 2010; Gordon *et al*. 2012; Rejmánek and Richardson 2013; El-Barougy *et al*. 2021; Guzmán *et al*. 2021). Weed Risk Assessment based on qualitative scores also classified it as presenting a moderate risk of invasion in Brazil (Ziller *et al*. 2018), and, in Portugal, it was included in an invasive species list (Marchante *et al*. 2014). Several authors, however, referred to it as a species with low dispersal outside planted areas, as studied in the United States (Ritter and Yost 2009; Callaham *et al*. 2013), Brazil (da Silva *et al*. 2011), Australia (Larcombe *et al*. 2013), and Portugal (Fernandes *et al*. 2016a). For Kirkpatrick (1977), the lack of mechanisms that enhance seed dispersal, which is crucial for expansion (Booth 2017), prevents *E. globulus* from being problematic regarding its invasive behaviour. In fact, the majority of seeds reached approximately only a horizontal distance equal to twice the height of the mother tree (Cremer 1977). Seeds fall by gravity, mainly within 15 m of trees (Calviño-Cancela and Rubido-Bará 2013), explaining the higher density of seedlings found inside plantations and within the first 15 m around plantation boundaries (Calviño-Cancela and Rubido-Bará 2013; Larcombe *et al*. 2013; Fernandes *et al*. 2016a; Águas *et al*. 2017a). Seedling establishment decreases exponentially with increasing distance to the mother tree (Larcombe *et al*. 2013; Fernandes *et al*. 2016a), and almost all seedlings (ca. 90%) are found 30 m away from plantation edge (Deus *et al*. 2019). A low density of seedlings was observed 175 m away from plantations, which was associated with a drainage line or windy conditions (Larcombe *et al*. 2013; Deus *et al*. 2019). However, various authors suggest that the naturalization of *E. globulus* is occurring in large part of Portuguese territory with favourable conditions for its development and establishment (Catry *et al*. 2015; Fernandes *et al*. 2016a; Águas *et al*. 2017a; Silva *et al*. 2021; Águas *et al*. 2022).

The development of the *E. globulus* tree is easily identifiable by a change in leaf colour: juveniles have a bluish colour (which gives the species its name), changing to a common green when it reaches maturity (Mount 1969). Sexual maturity occurs at three to four years (Jordan *et al*. 1999), but seed production is suppressed in dense plantations and does not occur until the trees are at least seven years old (Kirkpatrick 1975). Phenological development lasts about one year, from flower bud initiation to seed set. Flower bud maturation forms a flower (Hingston and Potts 1998), distinguished by the presence of an operculum composed by the fusion of sepals and petals (Fig. 1.3). The presence of this operculum gives the genus its name, derived from the Greek *eu*, “well”, and *calyptus*, “covered” (Eldridge *et al*. 1993).

Flowers are bisexual (i.e., male and female structures are present in the same flower) with a mixed mating system (Eldridge *et al*. 1993) and are receptive to bird and insect (mainly bees) pollination (Jacobs 1979). Pollen can fertilize flowers from the same tree, increasing inbreeding and lowering seed viability, survival, and offspring fertility (Hardner and Potts 1995; Hardner *et al*. 1996; Charlesworth and Willis 2009). To avoid self-fertilization, pollen is released a few days before the stigma becomes receptive, a mechanism called protandry (Jacobs 1979).

Fruits are woody capsules with triangular valves at the top (Eldridge *et al*. 1993) that change colour from green to brown as they mature (Jacobs 1979). Although capsules fall throughout the year, primarily in autumn and winter (Calviño-Cancela and Rubido-Bará 2013), *E. globulus* is a serotinous species since some capsules are retained in the canopy for a few years, forming a canopy seed bank (Mount 1969; Lamont *et al*. 1991). Each tree can produce hundreds of capsules, each containing ca. 6 to 25 viable seeds (Hardner and Potts 1995; Mimura *et al*. 2009) that have no dormancy and germinate under favourable conditions (Nereu *et al*. 2019).

Figure 1.3 Different phenological stages of *Eucalyptus globulus*, including a) flower bud, b) flower, c) to e) capsules in different maturation stages.

Despite having a strong capacity for adaptation to various climatic and soil conditions, *E. globulus* is still susceptible to biotic and abiotic stresses conditions (Stoneman 1994). Drought, for example, affects seed germination (González-Muñoz *et al*. 2011), and litter prevents radicle from obtaining nutrients from the soil (Calviño-Cancela *et al*. 2018), an important step since nutrients are obtained from the photosynthesis of early emerged cotyledons (Boland *et al*. 1980). Furthermore, low temperatures (Almeida *et al*. 1994; Águas *et al*. 2017a; Fernandes *et al*. 2017; Deus *et al*. 2019), reduced light availability (Fernandes *et al*. 2018), and interspecific competition (Garau *et al*. 2009) compromise seedling survival. In this sense, seedling establishment is reduced in forests and grasslands (Calviño-Cancela and Rubido-Bará 2013; Fernandes *et al*. 2018) but enhanced in disturbed areas (Fernandes *et al*. 2017), such as after forest fires (Águas *et al*. 2014, Calviño-Cancela *et al*. 2018) when seeds and seedlings can find favourable conditions to germinate and establish. Following this successful establishment, and if these seedlings grow and develop properly, there may be a shift in community diversity and functioning. Consequently, despite *E. globulus*'s crucial role in countries' economies, plantations negatively impact ecosystems due to changes in soil and water cycles (Castro-Díez *et al*. 2012; 2021), to the reduction of plant diversity (Calviño-Cancela *et al*. 2012; Vaz *et al*. 2019) through the recurrent management actions and allelopathic effects of litter on another plant establishment (Molina *et al.*1991; Souto *et al*. 2001; Carneiro *et al*. 2008; Águas *et al*. 2017b), and this change in community diversity and functioning leads to a shift in fire hazard (Wolf and DiTomaso 2015).

1.4 Forest fires and *Eucalyptus globulus* plantations

Forest fires have historically played a major role in the distribution and adaptation of plant communities (Pausas and Keeley 2009) and have been used as a land management tool by populations (Pausas and Vallejo 1999). Fire, considered as an important landscape driver, alters flora composition and structure (Silva *et al*. 2011), modifies soil physical and chemical properties (Litton and Santelices 2003), and increases erosion and fertility loss (Faria *et al*. 2015). Given the Mediterranean climate, characterized by mild winters and dry and hot summers (Cowling *et al*. 1996), fires have an ecological, economic, and social impact on southern Europe every year (Moreira *et al*. 2012). Portugal has the highest percentage of burnt area in Europe, making fire one of this country's most serious environmental issues (Mateus and Fernandes 2014; Turco *et al*. 2016).

Aside from weather conditions, vegetation structure and biomass accumulation affect forest fire progression particularly in dense and closed stands (Fernandes 2009). Moreover, forested areas occupied by fast-growing species in a monoculture regime are even more flammable and susceptible to fire (Fernandes *et al*. 2016b; Gómez-González *et al*. 2018), and the presence of exotic vegetation may alter the risk and spread of fire due to higher fuel quantities and flammability (Aslan *et al*. 2020). Furthermore, forest fires increase rural abandonment, which leads to more fuel accumulation and significantly increases the risk of fire occurrence (Rego and Silva 2014), perpetuating the vicious cycle.

The litter and bark of *E. globulus* are extremely flammable (Popović *et al*. 2021), increasing the fire risk (Moreira *et al*. 2009; Silva *et al*. 2009). In Portugal, eucalypt areas accounted for approximately 30% of the total burnt area (ICNF 2024); however, the burnt area did not increase with the expansion of planted area (Fernandes *et al*. 2019). In fact, tree species are less important than stand structure (Fernandes 2009), as dense monocultures increase crown fire spread (Goodrick and Stanturf 2012). Furthermore, fire ignition is lower in managed conditions because of the lack of shrubs and bark shedding, whereas unmanaged and abandoned plantations raise special concerns (Fernandes 2009; Fernandes *et al*. 2019).

Native to a fire-prone zone, *E. globulus* is highly adapted to post-fire regeneration (Larcombe *et al*. 2013; Águas *et al*. 2014; Calviño-Cancela *et al*. 2018), both by seed germination and resprout from dormant epicormic buds present in trunks and branches (Jacobs 1979), through the presence of lignotuber which supplies energy for tissues' regeneration (Whittock *et al*. 2003). The aerial part of the plant is severely damaged by fire, but basal resprouting reduces plant mortality (Catry *et al*. 2013). Heat reduces and inhibits seed germination (Arán *et al*. 2013), so seeds in the soil are affected even inside capsules (dos Santos *et al*. 2015). On the other hand, lower temperatures reaching the canopies and the moist living tissue in the capsules' constitution allow canopy seed bank protection (Mount 1969; Gill 1981; Silva *et al*. 2016). Following a fire, capsules in the canopy dehydrate, and valves open, releasing large amounts of seeds (dos Santos *et al*. 2015; Silva *et al*. 2016). Eucalypt seeds are short-lived (Battaglia 1996) and readily germinate in favourable conditions, such as bare soil and a lack of allelopathic substances (Águas *et al*. 2017b; Queirós *et al*. 2020), increased nutrient content (*via* mineralization of organic matter) and water availability (González-Muñoz *et al*. 2011), reduced interspecific competition (Adams *et al*. 2003) and light availability (Gill 1997; Fernandes *et al*. 2017). Thus, the occurrence of fire promotes the establishment and expansion of this species (Silva *et al*. 2011; Águas *et al*. 2014; Calviño-Cancela *et al*. 2018), both through very effective post-fire regeneration and increased invasibility potential of the disturbed ecosystem (Davis *et al*. 2000; Fernandes *et al*. 2017). In this case, removing existing vegetation reduces competition for water and nutrients, allowing for easier establishment and dispersal. Furthermore, flowering and seed production in trees on the plantation edge are higher (Barbour *et al*. 2008), increasing the likelihood of expansion beyond the planted area.

Over the last 50 years, the maximum temperature has risen, particularly in summer, increasing the number of hot days (temperatures above 35 ºC) (Pausas 2004; Turco *et al*. 2019; IPCC 2021) while precipitation and the rainy season have decreased (Miranda *et al*. 2002), resulting in greater fire danger (Mateus and Fernandes 2014). These conditions, combined with drier combustible materials, have favoured the occurrence of extreme forest fires, such as those that occurred in Chile (Gómez-González *et al*. 2018), Brazil (Barlow *et al*. 2020), and the United States and Canada in recent years (Schulze *et al*. 2020; Hu *et al*. 2024). As a result, fires are more severe and intense (Amatulli *et al*. 2013; Turco *et al*. 2019), and the fire season has been extended (Moriondo *et al*. 2006; Dupuy *et al*. 2020; Rodrigues *et al*. 2023).

Longer and warmer summers, as well as more frequent heat waves (Wang *et al*. 2021), have increased the number of fires occurring earlier in spring and lasting until late autumn (Castellnou *et al*. 2018; Sánchez-Benítez *et al*. 2018), outside of the months that the authorities consider normal. In 2017, two catastrophic fire events occurred in Portugal on the 17th of June (early in the fire season) and on the 15th of October (late in the season), with a total burnt area of approximately 52 000 and 190 000 ha, respectively (ICNF 2017), as a result of an unusually dry winter and spring (Turco *et al*. 2019) and decreased vegetation moisture (Sánchez-Benítez *et al*. 2018). These occurrences had a major impact on the population and the media, not only because of the large burnt area, but also due to the number of human losses.

Exotic species expansion and climate change could influence fire behaviour towards more intense and large fires (Aslan *et al*. 2020), ultimately altering plant communities and favouring invasive species expansion (Davis *et al*. 2000; Nolan *et al*. 2021). Although *E. globulus* post-fire natural regeneration is well known, this change in fire season may increase its dispersal potential because post-fire climatic conditions can also be ascribed to different fire dates.

Improved genetic tree material, which could reduce planted area without compromising yield, could be essential for a more efficient management of plantation forests. Tree improvement programs for *E. globulus* have been developed to promote growth, adaptability, and adequate wood properties. However, given the controversy surrounding this species' potential invasive behaviour, it is critical to investigate whether selecting phenotypes most suitable for pulp production will affect this species' reproductive ecology.

1.5 Tree improvement and phenological behaviour

It is well known that plantations with selected genotypes focusing on economic and ecological traits that improve growth, wood quality, and insect and disease tolerance (Karnosky 1981; Rezende *et al*. 2014) and shortening harvest cycles (Fox *et al*. 2007; Zalesny *et al*. 2011) increase productivity levels. However, although the benefits of vegetative propagation have been recognized (Wu 2019), constituting a millennium-old agricultural technique, forest tree species improvement is only about a century old (Neale and Kremer 2011). Moreover, unlike most crops, breeding programs for forest trees aim to improve existing populations rather than develop new ones (Neale and Kremer 2011) by selecting and crossing superior trees with desirable characteristics to produce offspring that outperform their parents (Potts *et al*. 2004). This procedure consists of 1) collecting seeds or cuttings after phenotypic selection of trees in natural stands, 2) breeding and establishing seed orchards with the best-performing trees, 3) controlled pollination of selected phenotypes and superior seed collection, 4) progeny testing based on offspring performance, and 5) seed orchard establishment to genetically-improved seed production. Finally, the improvement program is completed through the establishment of clonal plantations and the evaluation of individuals' field performance (White 1987; Sedjo 1999; Harfouche *et al*. 2012). Therefore, a commercial clone is "a group of genetically identical plants, produced by vegetative propagation from the same common ancestor, which was selected for its all-round outstanding performance" (Rezende *et al*. 2014). As a result, the best-adapted genotypes will be planted in a given area, resulting in higher yields and better meeting needs. The selection criteria vary by species, but the ultimate goal is to increase favourable traits related to productivity: increased growth and stem form, vigour, wood quality and pest and disease resistance (Kedharnath 1984).

Given the commercial importance of *E. globulus*, tree improvement programs have been developed to enhance desirable wood properties such as cellulose content, optimum fibre length, and increased pulp yield, as well as select individuals more resistant to adverse climatic conditions and pests (Potts *et al*. 2011). Furthermore, *Eucalyptus* species are ideal for breeding programs due to their ease of propagation, short generation intervals, and genetic variability (Zalesny *et al*. 2011). In Portugal, the pulp company Celbi initiated these programs in 1966 (Potts *et al*. 2004) based on local populations and provenances imported from Australia (Borralho *et al*. 1992). Other pulp companies (Portucel, Soporcel, and Caima) began their improvement programs in the 1980s and 1990s (Borralho *et al*. 2007). Nowadays, improved material-based forestry is mostly implemented by pulp companies through intensive clonal plantations (Almeida *et al*. 2005), and as a result, wood productivity has increased by 25 to 50% (Borralho *et al*. 2007; Rezende *et al*. 2014).

Aside from increasing yield, there are some indications that breeding programs may alter flowering and fruiting in *Eucalyptus* species (Moncur and Boland 2000; Varghese *et al*. 2009), which could affect the dispersal potential of improved trees. However, it remains unclear whether these breeding programs affect the phenological and reproductive behaviour of *E. globulus,* which is especially important in the exotic range, where there is a risk of expansion beyond plantation areas.

The impact of these breeding programs is particularly relevant since the phenological development of *E. globulus* is heritable (Chambers *et al*. 1997), and the time of first flowering is genetically controlled (Jordan *et al*. 1999). However, despite being a conservative trait, flowering onset is also influenced by abiotic factors such as temperature, moisture and nutrient availability and provenance (Eldridge *et al*. 1993), making research in introduced areas critical. Furthermore, external factors such as tree spacing, climatic conditions, and pollination effectiveness influence eucalypt seed production (Eldridge *et al*. 1993), whereas maternal genotype and seed size influence germination rate and seedling growth (Martins-Corder *et al*. 1997; Rix *et al*. 2015). Also, different landraces used in breeding programs have genetically variable flowering timing (Gore and Potts 1995) and different capsule and seed sizes (McGowen *et al*. 2004). Indeed, understanding the reproductive behaviour of these improved trees is critical in accounting for such a wide range of interactions with local conditions.

Improved materials are typically implemented in the same geographic region as their parents, where conditions are most favourable (Gray *et al*. 2016). On the other hand, climate change alters expected patterns, which may impact tree performance because the selection process reduces genetic variability, lowering adaptation to different conditions (Turnbull 1999). In this sense, changes in phenology have long been regarded as a sensitive indicator of climatic change (Bertin 2008). Finally, considering that seed ecology plays a critical role in species invasion (Rejmánek and Richardson 2011), the impact of tree improvement on phenological dynamics, fruit characteristics, and seed germination must be assessed to determine whether clonal plantations will slow or accelerate species dispersal. In this regard, new plantations must be closely monitored to ensure that trees do not expand outside plantations.

General aims and thesis outline

Eucalyptus globulus is a controversial species in its exotic range, particularly in Mediterranean-type climate regions where frequent forest fires are combined with the great ability of this species to regenerate. With the highest percentage of burnt area in Europe and *E. globulus* occupying a quarter of the forested area, Portugal has a particular interest for studying factors that influence the natural regeneration of this species. Furthermore, climate change has altered the fire occurrence, resulting in an extension of the fire season. In this regard, the off-season *E. globulus* post-fire regeneration dynamic is still unknown, which could significantly impact its dispersal since weather and local conditions may differ. This is especially important in unmanaged plantations, where there is a higher probability of natural regeneration after a fire, but also under large trees, where reproductive output is considerable. Implementing clonal plantations with selected individuals for increased wood production and quality may reduce occupied areas. However, these trees' phenological behaviour and production of reproductive structures must be studied since long-distance seed dispersal capacity is an essential step to identify invasion events and invasive species.

Recognizing the importance of *E. globulus* in Portugal as a representative forest species with high economic and industrial value and identifying drivers for its establishment is critical to contributing to a better forest management and expansion control protocols, as its potential invasiveness is a major concern. With that in mind, this thesis aims to understand (Fig. 1.4):

- Establishment, development, and dispersal success in the context of climate change and fire events through the impact of i) off-season fires, ii) plantations pre-fire management, iii) isolated seed-trees, and iv) local drivers;
- The phenological characteristics and dispersal dynamics of improved *Eucalyptus* clones, namely i) potential reproductive behaviour, ii) seed production, and iii) germination capacity, aiming to valorise clones as management tool to reduce planted area.

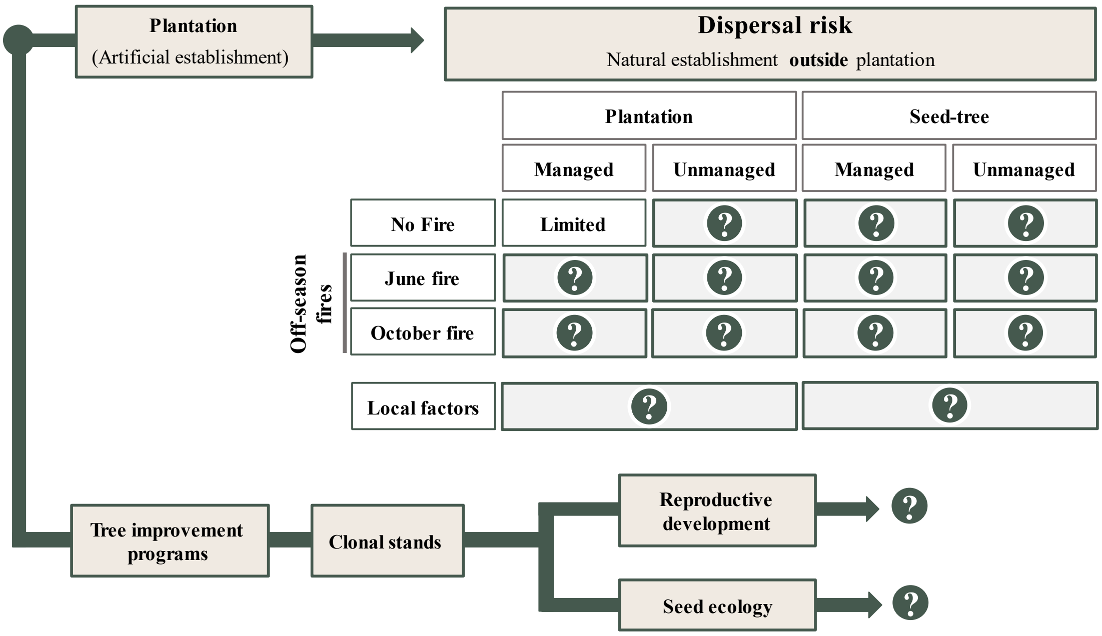


Figure 1.4 Main research questions addressed in this thesis, considering the current gaps in knowledge ascribed to *E. globulus* establishment and dispersal.

This thesis includes three scientific papers published in peer-reviewed international journals, and one paper in preparation, each corresponding to a chapter. Each chapter's specific objectives and main methodological approaches are described below:

Chapter 1. General introduction, aims and thesis outline

In this chapter, *E. globulus* main characteristics are contextualized, emphasizing its socioeconomic role around the world. A general description is also provided, focusing on its main characteristics, phenological behaviour, and factors influencing its natural regeneration, such as forest fires. Furthermore, the controversy over *E. globulus*' invasive potential is addressed, raising concerns about the impact of off-season fires, pre-fire management, the presence of seed-trees, and tree improvement on this exotic species dispersal in Portugal.

Chapter 2. Management and fire, a critical combination for *Eucalyptus globulus* dispersal

Fire promotes the natural regeneration of *E. globulus*, which potentially threatens introduced ecosystems given this species ability to spread. Thus, assessing this species' post-fire dynamics is critical with the extension of the fire season caused by global warming. Therefore, the main goal of this chapter was to evaluate the impact of off-season fires on the success of *E. globulus* recruitment and dispersal, as well as the role of pre-fire management and large isolated trees as a possible key propagule source. To achieve this, the risk of *E. globulus* dispersal was studied by i) assessing sapling establishment outside plantation edges and under seed-trees in the absence of fire and after two fire events (June and October 2017, early and late in the season, respectively), ii) analysing the role of vegetation management actions prior to a fire in establishment outside plantation boundaries and in surrounding areas of seed-trees, and iii) evaluating sapling survival rate two years after the fires.

Chapter 3. Climate and local factors influence *Eucalyptus globulus* establishment after off-season fires

Despite its wide adaptation, *E. globulus* establishment is influenced by biotic and abiotic stresses that may act as critical barriers to its invasiveness behaviour. As a result, research focusing on local factors is relevant, especially in the context of off-season fires. Therefore, how *E. globulus* saplings respond to climate and local conditions in plantations and under seed-trees was studied following the 2017 June and October fire events. In this regard, the impact of post-fire conditions after the two fire events on the establishment of *E. globulus* saplings was assessed by evaluating the climatic and local conditions affecting the natural regeneration in managed and unmanaged plantations and under seed-trees.

Chapter 4. Exploring reproductive biology in *Eucalyptus globulus* clones under distinct climatic conditions

Given the economic importance of *E. globulus*, improved material-based clonal forestry is being implemented to increase productivity. However, it is unclear whether the tree's reproductive dynamics will change due to these improvement programs. In this sense, the phenological behaviour of clonal-based trees was studied by comparing i) the timing and duration of reproductive stages and ii) the number of reproductive structures produced in three clonal and seedling-based stands in two climate-contrasting sites (mesic *vs* xeric) in Portugal.

Chapter 5. From capsules to germination: an assessment of *Eucalyptus globulus* dispersal mechanisms

In the current context of expanding plantation areas using improved material-based trees along with the presence of isolated seed-trees, and considering reproductive traits as the major driver of the invasive process, it is critical to understand the impact of improvement programs on *E. globulus* seed characteristics. With that in mind, the characteristics related to the reproductive biology of clonal trees (improved trees) and seed-trees were compared with unimproved seedling-based trees. During a year, capsules were collected from clonal and seedling-based trees, as well as from seed-trees, in two climate-contrasting sites (mesic *vs* xeric) in Portugal. The collected capsules were characterized regarding their time since production (2018 to 2021), size, weight, and number of seeds; subsequently, germination capacity of these seeds was assessed.

Chapter 6. General discussion and main conclusions

In this last section, the main conclusions gathered from the preceding chapters are summarized, along with their implications for forestry management aiming to prevent *E. globulus* dispersal into natural habitats beyond plantation areas.

References

Adams P, Beadle C, Mendham N, Smethurst PJ (2003) The impact of timing and duration of grass control on growth of a young *Eucalyptus globulus* Labill. plantation. New For 26:147-165. https://doi.org/10.1023/A:1024490707175

Águas A, Ferreira A, Maia P, Fernandes PM, Roxo L, Keizer J, Silva JS, Rego FC, Moreira F (2014) Natural establishment of *Eucalyptus globulus* Labill. in burnt stands in Portugal. For Eco Man 323:47-56. https://doi.org/10.1016/j.foreco.2014.03.012

Águas A, Larcombe MJ, Matias H, Deus E, Potts BM, Rego FC, Silva JS (2017a) Understanding the naturalization of *Eucalyptus globulus* in Portugal: a comparison with Australian plantations. Eur J Forest Res 136:433-446. https://doi.org/10.1007/s10342-017-1043-6

Águas A, Incerti G, Saracino A, Lanzotti V, Silva JS, Rego FC, Mazzoleni S, Bonanomi G (2017b) Fire effects on litter chemistry and early development of *Eucalyptus globulus*. Plant Soil 422-514. https://doi.org/10.1007/s11104-017-3419-2

Águas A, Matias H, Rodrigues A, Bailey T, Silva J, Rego F (2022) Microsite drivers of natural seed regeneration of *Eucalyptus globulus* Labill. in burnt plantations. Forests 13(6):889. https://doi.org/10.3390/f13060889

Almeida MH, Chaves MM, Silva JC (1994) Cold acclimation in eucalypt hybrids. Tree Phys 14:921-932. https://doi.org/10.1093/treephys/14.7-8-9.921

Almeida MH, Araújo C, Araújo JA, Costa e Silva F, Neves I, Paiva V, Santiago A, Ribeiro D (2005) Melhoramento genético do eucalipto: que impacto na realidade? In: Silva R, Páscoa F (eds) A Floresta e as gentes, 5° Congresso Florestal Nacional. Sociedade Portuguesa de Ciências Florestais, Viseu, pp 145-146.

Alves AM, Pereira JS, Silva JN (2007) O eucaliptal em Portugal - Impactes ambientais e investigação científica. ISAPress, Lisbon

Amatulli G, Camia A, San-Miguel-Ayanz J (2013) Estimating future burned areas under changing climate in the EU-Mediterranean countries. Sci Total Environ 450-451:209-222. https://doi.org/10.1016/j.scitotenv.2013.02.014

Arán D, García-Duro J, Reyes O, Casal M (2013) Fire and invasive species: modifications in the germination potential of *Acacia melanoxylon*, *Conyza canadensis* and *Eucalyptus globulus*. For Eco Man 302:7-13. https://doi.org/10.1016/j.foreco.2013.02.030

Aslan CE, Dickson BG (2020) Non-native plants exert strong but under-studied influence on fire dynamics. NeoBiota 61:47-64. https://doi.org/10.3897/neobiota.61.51141

Badalamenti E, Cusimano D, Mantia TL, Pasta S, Romano S, Troia A, Vincenzo I (2018) The ongoing naturalisation of *Eucalyptus* spp. in the Mediterranean Basin: New threats to native species and habitats. Aust For 81(4):239-249. https://doi.org/10.1080/00049158.2018. 1533512

Barbour RC, Otahal Y, Vaillancourt RE, Potts BM (2008) Assessing the risk of pollen-mediated gene flow from exotic *Eucalyptus globulus* plantations into native eucalypt populations of Australia. Biol Conserv 141:896-907. https://doi.org/10.1016/j.biocon.2007.12.016

Barlow J, Berenguer E, Carmenta R, França, F (2020) Clarifying Amazonia's burning crisis. Glob Change Biol 26:319-321. https://doi.org/10.1111/gcb.14872

Battaglia M (1996) Effects of seed dormancy and emergence time on the survival and early growth of *Eucalyptus delegatensis* and *E. amygdalina*. Aust J Bot 44:123-137. https://doi.org/10.1071/BT9960123

Benali A, Sá A, Pinho J, Fernandes P, Pereira J (2021) Understanding the impact of different landscape-level fuel management strategies on wildfire hazard. Forests 12(5):522. https://doi/10.3390/f12050522

Bertin RI (2008) Plant phenology and distribution in relation to recent climate change. J Torrey Bot Soc 135(1):126-146. http://doi.org/10.3159/07-RP-035R.1

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26(7):333-339. https://doi/10.1016/j.tree.2011.03.023

Boland DJ, Brooker MIH, Turnbull JW (1980) *Eucalyptus* seed. CSIRO publishing, Canberra

Booth TH (2013) Eucalypt plantations and climate change. For Eco Man 301:28-34. https://doi:10.1016/j.foreco.2012.04.004

Booth TH (2017) Going nowhere fast: a review of seed dispersal in eucalypts. Aust J Bot 65:401-410. https://doi.org/10.1071/BT17019

Borralho N, Cotterill PP, Kanowski PJ (1992) Genetic control of growth of *Eucalyptus globulus* in Portugal. II. Efficiencies of early selection. Silvae Genet 41:70-77

Borralho NMG, Almeida MH, Potts BM (2007). O melhoramento do eucalipto em Portugal. In: Alves AM, Pereira JS, Silva JMN (eds) O eucaliptal em Portugal - Impactes ambientais e investigação científica. ISAPress, Lisbon, pp 61-110

Brundu G, Pauchard A, Pyšek P, Pergl J, Bindewald AM, Brunori A, Canavan S, Campagnaro T, Celesti-Grapow L, Dechoum MS, Dufour-Dror JM, Essl F, Flory SL, Genovesi P, Guarino F, Guangzhe L, Hulme PE, Jäger H, Kettle CJ, Krumm F, Langdon B, Lapin K, Lozano V, Le Roux JJ, Novoa A, Nuñez MA, Porté AJ, Silva JS, Schaffner U, Sitzia T, Tanner R, Tshidada N, Vítková M, Westergren M, Wilson JRU, Richardson DM (2020) Global guidelines for the sustainable use of non-native trees to prevent tree invasions and mitigate their negative impacts. NeoBiota 61:65-116. https://doi.org/10.3897/neobiota.61.58380

Brus R, Pötzelsberger E, Lapin K, Brundu G, Orazio C, Straigyte L, Hasenauer H (2019) Extent, distribution and origin of non-native forest tree species in Europe. Scand J For Res 34(7):1-12. https://doi.org/10.1080/02827581.2019.1676464

Byrne M (2008) Phylogeny, diversity and evolution of eucalypts. In: Sharma AK, Sharma A (eds) Plant genome: Biodiversity and evolution. Science Publishers, pp 303-346

Callaham MA, Stanturf JA, Hammond WJ, Rockwood DL, Wenk ES, O'Brien JJ (2013) Survey to evaluate escape of *Eucalyptus* spp. seedlings from plantations in Southeastern USA. Int J For Res 2013:946374. https://doi.org/10.1155/2013/946374

Calviño-Cancela M, Rubido-Bará M, Van Etten E (2012) Do eucalypt plantations provide habitat for native forest biodiversity? For Eco Man 270:153-162. https://doi.org/10.1016/j.foreco.2012.01.019

Calviño-Cancela M, Rubido-Bará M (2013) Invasive potential of *Eucalyptus globulus*: Seed dispersal, seedling recruitment and survival in habitats surrounding plantations. For Eco Man 305:129-137. https://doi.org/10.1016/j.foreco.2013.05.037

Calviño-Cancela M, Chas-Amil ML, García-Martínez ED, Touza J (2016) Wildfire risk associated with different vegetation types within and outside wildland-urban interfaces. For Eco Man 372:1-9. https://doi.org/10.1016/ j.foreco.2016.04.002

Calviño-Cancela M, Lorenzo P, González L (2018) Fire increases *Eucalyptus globulus* seedling recruitment in forested habitats: Effects of litter, shade and burnt soil on seedling emergence and survival. For Eco Man 409:826-834. https://doi.org/10.1016/j.foreco.2017.12.018

Carneiro M, Fabião A, Martins MC, Silva MA, Hilário L, Lousã M, Madeira M (2008) Effects of harrowing and fertilisation on understory vegetation and timber production of a *Eucalyptus globulus* Labill. plantation in Central Portugal. For Eco Man 255:591-597. https://doi.org/10.1016/J.FORECO.2007.09.028

Carnus JM, Parrotta J, Brockerhoff EG, Arbez M, Jactel H, Kremer A, Lamb D, O'Hara K, Walters B (2006) Planted forests and biodiversity. J For 104(2):65-77. https://doi.org/10.1093/jof/104.2.65

Castellnou M, Guiomar N, Rego F, Fernandes P (2018) Fire growth patterns in the 2018 mega fire episode of October 15, central Portugal. Adv For Fire Res 447-453. https://doi.org/10.14195/978-989-26-16-506_48

Castro G, Teixeira D, Ares-Pereira G, Lima C, Magalhães AR, Camarinha C, Guillera-Arroita G, Fonseca C, Rosalino L (2022) Drivers of occupancy patterns for the red fox, *Vulpes vulpes*, in Mediterranean *Eucalyptus* plantations. For Eco Man 519:120293. https://doi.org/10.1016/j.foreco.2022.120293

Castro-Díez P, Fierro-Brunnenmeister N, González-Muñoz N, Gallardo A (2012) Effects of exotic and native tree leaf litter on soil properties of two contrasting sites in the Iberian Peninsula. Plant Soil 350:179-191. https://10.1007/s11104-011-0893-9

Castro-Díez P, Alonso Á, Saldaña-López A, Granda E (2021) Effects of widespread non-native trees on regulating ecosystem services. Sci Total Environ 778:146141. https://doi.org/10.1016/j.scitotenv.2021.146141

Catry FX, Moreira F, Tujeira R, Silva JS (2013) Post-fire survival and regeneration of *Eucalyptus globulus* in forest plantations in Portugal. For Eco Man 310:194-203. http://doi.org/10.1016/j.foreco.2013.08.036

Catry FX, Moreira F, Deus E, Silva JS, Águas A (2015) Assessing the extent and the environmental drivers of *Eucalyptus globulus* wildling establishment in Portugal: results from a countrywide survey. Biol. Invasions 17:3163-3181. https://doi.org/10.1007/s10530-015-0943-y

Cerasoli S, Caldeira MC, Pereira JS, Caudullo G, de Rigo D (2016) *Eucalyptus globulus* and other eucalypts in Europe: Distribution, habitat, usage and threats. In: San-Miguel-Ayanz J, de Rigo D, Caudullo G, Houston Durrant T, Mauri A (eds) European atlas of forest tree species. Publ. Off. EU, Luxembourg, pp 90-91

Chambers PGS, Potts B, Tilyard P (1997). The genetic control of flowering precocity in *Eucalyptus globulus* ssp. *globulus*. University of Tasmania. Journal contribution 46(4). https://hdl.handle.net/102.100.100/491181

Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. Nat Rev Genet 10(11):783-96. https://doi.org/10.1038/nrg2664

Cowling RM, Rundel PW, Lamont BB, Arroyo MK, Arianoutsou M (1996) Plant diversity in Mediterranean-climate regions. Trends Ecol Evol 11(9):362-366. https://doi.org/10.1016/0169-5347(96)10044-6

Cremer KW (1977) Distance of seed dispersal in eucalypts estimated from seed weights. Aust For 7:225-228

da Silva PHM, Poggiani F, Sebbenn A, Mori E (2011) Can *Eucalyptus* invade native forest fragments close to commercial stands. For Eco Man 261:2075-2080. https://doi.org/10.1016/j.foreco.2011.03.001

da Silva PHM, Bouillet J-P, Paula RC (2016) Assessing the invasive potential of commercial *Eucalyptus* species in Brazil: Germination and early establishment. For Eco Man 374:129-135. https://doi.org/10.1016/j.foreco.2016.05.007

Daba M (2016) The *Eucalyptus* dilemma: The pursuit for socio-economic benefit versus environmental impacts of *Eucalyptus* in Ethiopia. J Nat Sci Res 6:127-137

Davis M, Grime J, Thompson K (2000) Fluctuating resources in plant communities: A general theory of invasibility. J Ecol 88:528-536. https://doi.org/10.1046/j.1365-2745.2000.00473.x

Dawson W, Burslem DFRP, Hulme PE (2009) Factors explaining alien plant invasion success in a tropical ecosystem differ at each stage of invasion. J Ecol 97(4):657-665. https://doi.org/10.1111/j.1365-2745.2009.01519.x

Deus E, Silva JS, Larcombe MJ, Catry FX, Queirós L, dos Santos P, Matias H, Águas A, Rego FC (2019) Investigating the invasiveness of *Eucalyptus globulus* in Portugal: Site-scale drivers, reproductive capacity and dispersal potential. Biol Invasions 21:2027-2044. https://doi.org/10.1007/s10530-019-01954-6

Dodet M, Collet C (2012) When should exotic forest plantation tree species be considered as an invasive threat and how should we treat them? Biol Invasions 14:1765-1778. https://doi.org/10.1007/s10530-012-0202-4

dos Santos P, Matias H, Deus E, Águas A, Silva JS (2015) Fire effects on capsules and encapsulated seeds from *Eucalyptus globulus* in Portugal. Plant Ecol 216:1611-1621. https://doi.org/10.1007/s11258-015-0544-y

Dupuy Jl, Fargeon H, Martin-StPaul N, Pimont F., Ruffault J, Guijarro M, Hernando C, Madrigal J, Fernandes P (2020) Climate change impact on future wildfire danger and activity in southern Europe: A review. Ann For Sci 77:35. https://doi.org/10.1007/s13595-020-00933-5

Dutkowski GW, Potts BM (1999) Geographic patterns of genetic variation in *Eucalyptus globulus* ssp. *globulus* and a revised racial classification. Aust J Bot 47:237-263. https://doi.org/10.1071/BT97114

Dyck B (2003) Benefits of planted forests: Social, ecological and economic. Inter-sessional expert meeting on the role of planted forests in sustainable forest management. New Zealand, 25-27

El-Barougy R, Dakhil M, Halmy M, Gray S, Abdelaal M, Khedr A, Bersier LF (2021) Invasion risk assessment using trait-environment and species distribution modelling techniques in an arid protected area: Towards conservation prioritization. Ecol Indic 129:107951. https://doi.org/10.1016/j.ecolind.2021.107951

Eldridge K, Davidson J, Harwood C, Van Wyk G (1993) Eucalypt domestication and breeding. Claredon Press, Oxford

Essl F, Moser D, Dullinger S, Mang T, Hulme PE (2010) Selection for commercial forestry determines global patterns of alien conifer invasions. Divers Distrib 16:911-921. https://doi.org/10.1111/j.1472-4642.2010.00705.xC

FAO. 2020. Global forest resources assessment 2020: Main report. Rome

Faria SR, De la Rosa JM, Knicker H, González-Pérez JA, Keizer JJ (2015) Molecular characterization of wildfire impacts on organic matter in eroded sediments and topsoil in Mediterranean eucalypt stands. Catena 135:29-37. https://doi.org/10.1016/j.catena.2015.07.007

Feliciano D, Alves R, Mendes A, Ribeiro M, Sottomayor M (2015) Forest Land Ownership Change in Portugal. COST Action FP1201 FACESMAP Country Report, European Forest Institute Central-East and South-East European Regional Office, Vienna. 50 pages. [Online publication]

Fernandes PM (2009) Combining forest structure data and fuel modelling to assess fire hazard in Portugal. Ann For Sci 66:415. https://doi.org/10.1051/forest/2009013

Fernandes PM, Loureiro C, Palheiro P, Vale-Gonçalves H, Fernandes MM, Cruz MG (2011) Fuels and fire hazard in blue gum (*Eucalyptus globulus*) stands in Portugal. Boletín del CIDEU 10:53-61

Fernandes P, Antunes C, Pinho P, Máguas C, Correia O (2016a) Natural regeneration of *Pinus pinaster* and *Eucalyptus globulus* from plantation into adjacent natural habitats. For Ecol Man 378:91-102. https://doi.org/10.1016/j.foreco.2016.07.027

Fernandes PM, Barros AMG, Pinto A, Santos JA (2016b) Characteristics and controls of extremely large wildfires in the western Mediterranean Basin. J Geophys Res Biogeosci 121:2141-2157. https://doi.org/10.1002/2016JG003389

Fernandes P, Máguas C, Correia O (2017) Combined effects of climate, habitat, and disturbance on seedling establishment of *Pinus pinaster* and *Eucalyptus globulus*. Plant Ecol 218(5):501-515. https://doi.org/10.1007/s11258-017-0706-1

Fernandes P, Máguas C, Correia O, González-Moreno P (2018) What drives *Eucalyptus globulus* natural establishment outside plantations? The relative importance of climate, plantation and site characteristics. Biol Invasions 20:1129-1146. https://doi.org/10.1007/s10530-017-1614-y

Fernandes PM, Guiomar N, Rossa CG (2019) Analysing eucalypt expansion in Portugal as a fire-regime modifier. Sci Total Environ 666:79-88. https://doi.org/10.1016/j.scitotenv.2019.02.237

Ferreira V, Boyero L, Calvo C, Correa F, Figueroa R, Goncalves Jr JF, Goyenola G, Graça MAS, Hepp LU, Kariuki S, López-Rodríguez A, Mazzeo N, M'Erimba C, Monroy S, Peil A, Pozo J, Rezende R, Teixeira-de-Mello Franco F (2019) A Global assessment of the effects of *Eucalyptus* plantations on stream ecosystem functioning. Ecosystems 22:629-642. https://doi.org/10.1007/s10021-018-0292-7

Forsyth GG, Richardson DM, Brown PJ, van Wilgen BW (2004) A rapid assessment of the invasive status of *Eucalyptus* species in two South African provinces. S Afr J Sci 100:75-77. http://hdl.handle.net/10204/2173

Fox T, Jokela E, Allen H (2007) The development of pine plantation silviculture in the Southern United States. J For 105:337-347. https://doi.org/10.1093/jof/105.7.337

Garau AM, Ghersa CM, Lemcoff JH, Barañao JJ (2009) Weeds in *Eucalyptus globulus* subsp. *maidenii* (F. Muell) establishment: effects of competition on sapling growth and survivorship. New For 37:251-264. https://doi.org/10.1007/s11056-008-9121-8

Gassó N, Basnou C, Vilà M (2010) Predicting plant invaders in the Mediterranean through a weed risk assessment system. Biol Invasions 12:463-476. https://doi.org/10.1007/s10530-009-9451-2

Giam X, Bradshaw C, Tan H, Sodhi N (2010) Future habitat loss and the conservation of plant biodiversity. Biol Conserv 143:1594-1602. https://doi.org/10.1016/j.biocon.2010. 04.019

Gill AM (1981) Adapted response of Australian vascular plant species to fires. In: Gill AM, Groves RH, Noble IR (eds) Fire and the Australian biota. Australian Academy of Science, Canberra, pp 243-271

Gill AM (1997) Eucalypts and fire: interdependent or independent? In: Williams JE, Woinarski JCZ (eds) Eucalypt ecology. Cambridge University Press, Cambridge, pp 151-1167

Goded S, Ekroos J, Domínguez J, Azcárate JG, Guitián JA, Smith HG (2019) Effects of *Eucalyptus* plantations on avian and herb species richness and composition in North-West Spain. Glob Ecol Conserv 19:e00690. https://doi.org/10.1016/ j.gecco.2019.e00690

Goes E (1977) Os eucaliptos: Ecologia, cultura, produções e rentabilidade. Lisboa: Portucel

Golivets M, Wallin KF (2018) Neighbour tolerance, not suppression, provides competitive advantage to non-native plants. Ecol Lett 21:745-759. https://doi.org/10.1111/ele.12934

Gómez-González S, Ojeda F, Fernandes PM (2018) Portugal and Chile: Longing for sustainable forestry while rising from the ashes. Environ Sci Policy 81:104-107. https://doi.org/10.1016/j.envsci.2017.11.006

González-Muñoz N, Castro-Díez P, Fierro-Brunnenmeister N (2011) Establishment success of coexisting native and exotic trees under an experimental gradient of irradiance and soil moisture. Environ Manage 48:764-773. https://doi.org/10.1007/s00267-011-9731-3

Goodrick S, Stanturf J (2012) Evaluating potential changes in fire risk from *Eucalyptus* plantings in the Southern United States. Int J For Res 680246. https://doi.org/10.1155/2012/680246

Gordon DR, Flory SL, Cooper AL, Morris SK (2012) Assessing the invasion risk of *Eucalyptus* in the United States using the Australian weed risk assessment. Int J For Res 2012:203768. https://doi.org/10.1155/2012/203768

Gore PL, Potts B (1995) The genetic control of flowering time in *Eucalyptus globulus*, *E. nitens* and their F1 hybrids. University of Tasmania. Conference contribution. https://hdl.handle.net/102.100.100/493311

Gray L, Hamann A, John S, Rweyongeza D, Barnhardt L, Thomas B (2016) Climate change risk management in tree improvement programs: selection and movement of genotypes. Tree Genet. Genomes 12. https://doi.org/10.1007/s11295-016-0983-1

Guzmán S, Drummond J, Barreto C (2021) Undesirable neighbours: *Eucalyptus* and protected areas. In: Suratman MN (ed) Protected area management - Recent advances. IntechOpen. https://doi.org/10.5772/intechopen.99831

Henderson L (2001) Alien weeds and invasive plants: a complete guide to declared weeds and invaders in South Africa. Plant Protection Research Institute South Africa

ICNF (2017) 10º Relatório provisório de incêndios florestais. Departamento de Gestão de Áreas Públicas e de Protecção Florestal do Instituto da Conservação da Natureza e das Florestas. RIF102017

ICNF (2019) IFN6 – Anexo Técnico. [pdf], 31 pp, versão 1.0 Instituto da Conservação da Natureza e das Florestas, Lisboa

ICNF (2024) Áreas ardidas vs Uso e ocupação do solo 1996-2023. www.icnf.pt/florestas/gfr/gfrgestaoinformacao/estatisticas (accessed in June 2024)

IPCC (2021) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. In Press

Hardner CM, Potts BM (1995) Inbreeding depression and changes in variation after selfing in *Eucalyptus globulus* spp. *globulus*. Silvae Genet 44:46-54

Hardner CM, Vaillancourt RE, Potts BM (1996) Stand density influences outcrossing rate and growth of open-pollinated families of *Eucalyptus globulus*. Silvae Genet 45:226-228

Harfouche A, Meilan R, Kirst M, Morgante M, Boerjan W, Sabatti M, Scarascia Mugnozza G (2012) Accelerating the domestication of forest trees in a changing world. Trends Plant Sci 17:64-72. https://doi.org/10.1016/j.tplants.2011.11.005

Hingston AB, Potts BM (1998) Floral visitors of *Eucalyptus globulus* subsp. *globulus* in eastern Tasmania. Tas forests 10:125-40

Hu Y, Yue X, Tian C (2024) Climatic drivers of the Canadian wildfire episode in 2023. Atmos Ocean Sci Lett 17(4): 100483. https://doi.org/10.1016/j.aosl.2024.100483

Iglesias Trabado G, Wilstermann D (2009) *Eucalyptus* Universalis. Global cultivated eucalypt forests map 2009, GIT Forestry Consulting's Eucalyptologics: Information resources on *Eucalyptus* cultivation worldwide. http://www.git.forestry.com/ (accessed in June 2021)

Jacobs MR (1979) Eucalypts for planting. FAO, Rome

Jordan G, Potts BM, Wiltshire R (1999) Strong, independent quantitative genetic control of vegetative phase change and first flowering in *Eucalyptus globulus* ssp. *globulus*. Heredity (Edinb) 83:179-187. https://doi.org/10.1046/j.1365-2540.1999.00570.x

Karnosky DF (1981) Potential for forest tree improvement *via* tissue culture. BioScience 31(2):114-120. https://doi.org/10.2307/1308253

Kedharnath S (1984) Forest tree improvement in India. Proc Indian Acad Sci 93:401-412. https://doi.org/10.1007/BF03053091

Kirkpatrick JB (1975) Natural distribution of *Eucalyptus globulus* Labill. Aust Geogr 13:22-35

Kirkpatrick JB (1977) Eucalypt invasion in Southern California. Aust Geogr 13:387-393

Lake JC, Leishman MR (2004) Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. Biol Conserv 117:215-226. https://doi.org/10.1016/S0006-3207(03)00294-5

Lambdon PW, Lloret F, Hulme PE (2008) How do introduction characteristics influence the invasion success of Mediterranean alien plants? Perspec Plant Ecol Evol Syst 10:143-159. https://doi.org/10.1016/j.ppees.2007.12.004

Lamont BB, LeMaitre DC, Cowling RM, Enright NJ (1991) Canopy seed storage in woody plants. Bot Rev 57:277-317. https://doi.org/10.1007/BF02858770

Larcombe MJ, Silva JS, Vaillancourt RE, Potts BM (2013) Assessing the invasive potential of *Eucalyptus globulus* in Australia: Quantification of wildling establishment from plantations. Biol Invasions 15:2763-2781. https://doi.org/10.1007/s10530-013-0492-1

Levia DF, Creed IF, Hannah DM, Nanko K, Boyer EW, Carlyle-Moses DE, van de Giesen N, Grasso D, Guswa AJ, Hudson JE, Hudson SA, Iida S, Jackson RB, Katul GG, Kumagai T, Llorens P, Ribeiro FP, Pataki DE, Peters CA, Carretero DS, Selker JS, Tetzlaff D, Zalewski M, Bruen M (2020) Homogenization of the terrestrial water cycle. Nat Geosci 13:656-658. https://doi.org/10.1038/s41561-020-0641-y

Liao C, Luo Y, Fang C, Chen J, Li B (2012) The effects of plantation practice on soil properties based on the comparison between natural and planted forests: a meta-analysis. Glob Ecol Biogeogr 21:318-327. https://doi.org/10.1111/j.1466-8238. 2011.00690.x

Litton C, Santelices R (2003) Effect of wildfire on soil physical and chemical properties in a *Nothofagus glauca* forest, Chile. Rev Chi Hist Nat 76:529-542. https://doi.org/10.4067/S0716-078X2003000400001

Liu C, Kuchma O, Krutovsky K (2018) Mixed-species versus monocultures in plantation forestry: Development, benefits, ecosystem services and perspectives for the future. Glob Ecol Conserv 15:e00419. https://doi.org/10.1016/j.gecco.2018.e00419

Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. Trends Ecol Evol 20(5):223-228. https://doi:10.1016/j.tree. 2005.02.004

Lonsdale WM (1999) Global patterns of plant invasions and the concept of invasibility. Ecology 80(5):1522-1536. https://doi.org/10.2307/176544

Lorentz KA, Minogue PJ (2015) Potential invasiveness for *Eucalyptus* species in Florida. Inv Plant Sci Manag 8(1):90-97. https://doi.org/10.1614/IPSM-D-14-00030.1

Mallen-Cooper M, Atkinson J, Xirocostas ZA, Wijas B, Chiarenza GM, Dadzie FA, Eldridge DJ (2022) Global synthesis reveals strong multifaceted effects of eucalypts on soils. Glob Ecol Biogeogr 31:1667-1678. https://doi.org/10.1111/geb.13522

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

Martins-Corder MP, Mori ES, Carvalho MTV, Derbyshire E (1998) Genetic diversity of three size classes of seeds of *Eucalyptus globulus* ssp. *globulus*. Silvae Genet 47(1):6-14. http://hdl.handle.net/11449/219211

Mateus P, Fernandes P (2014) Chapter 4 - Forest fires in Portugal: dynamics, causes and policies. In: Reboredo F (ed) Forest context and policies in Portugal, present and future challenges. World Forests series, Springer, pp 97-114

McGowen M, Potts B, Vaillancourt R, Gore P, Williams D, Pilbeam D (2004) The genetic control of sexual reproduction in *Eucalyptus globulus* In: Borralho N, Pereira JS, Marques C, Coutinho J, Madeira M, Tomé M (eds) *Eucalyptus* in a changing world. Proc. of IUFRO Conf., Aveiro 11-15 October 2004

Messier C, Bauhus J, Sousa-Silva R, Auge H, Baeten L, Barsoum N, Bruelheide H, Caldwell B, Cavender-Bares J, Dhiedt E, Eisenhauer N, Ganade G, Gravel D, Guillemot J, Hall J, Hector A, Hérault B, Jactel H, Koricheva J, Zemp D (2021) For the sake of resilience and multifunctionality, let’s diversify planted forests! Conserv Lett 15:e12829. https://doi.org/10.1111/conl.12829

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: Synthesis. Island Press, Washington, DC

Miller KM, McGill BJ (2017) Land use and life history limit migration capacity of eastern tree species. Glob Ecol Biogeogr 27:57-67. https://doi.org/10.1111/geb.12671

Mimura M, Barbour R, Potts B, Vaillancourt R, Watanabe KN (2009) Comparison of contemporary mating patterns in continuous and fragmented *Eucalyptus globulus* native forests. Mol Ecol 18:4180-4192. https://doi.org/10.1111/j.1365-294X.2009.04350.x

Miranda PMA, Coelho FES, Tomé AR, Valente MA, Carvalho A, Pires C, Pires HO, Pires VC, Ramalho C (2002) 20th century Portuguese climate and climate scenarios. In: Santos FD, Forbes K, Moita R (eds) Climate change in Portugal: Scenarios, Impacts and Adaptation Measures (SIAM Project). Gradiva, pp 23-83

Molina A, Reigosa MJ, Carballeira A (1991) Release of allelochemical agents from litter, throughfall, and topsoil in plantations of *Eucalyptus globulus* Labill. in Spain. J Chem Eco 1(17). https://doi.org/10.1007/BF00994428

Moncur MW, Boland DJ (2000) Production of genetically improved *Eucalyptus nitens* seed for reforestation. Aust For 63(3):211-217. https://doi.org/10.1080/00049158.2000.10674833

Moreira F, Vaz P, Catry F, Silva J (2009) Regional variations in wildfire susceptibility of land-cover types in Portugal: Implications for landscape management to minimize fire hazard. Int J Wildland Fire 18:563-574. https:// doi.org/10.1071/WF07098

Moreira F, Arianoutsou M, Vallejo VR, las Heras J, Corona P, Xanthopoulos G, Fernandes P, Papageorgiou K (2012) Setting the scene for post-fire management. In: Moreira F, Arianoutsou M, Corona P, De las Heras J (eds) Post-fire management and restoration of Southern European forests. Managing Forest Ecosystems, Springer. https://doi.org/10.1007/978-94-007-2208-8_1

Moriondo M, Good P, Durão R, Bindi M, Giannakopoulos C, Corte-Real J (2006) Potential impact of climate change on fire risk in the Mediterranean area. Clim Res 31:85-95. https://doi.org/10.3354/cr031085

Mount AB (1969) Eucalypt ecology as related to fire. Proceedings of Tall Timbers Fire Ecology Conference 9:75-108

Nair KSS (2001) Pest outbreaks in tropical forest plantations: Is there a greater risk for exotic tree species? Centre for International Forestry Research, Jakarta, Indonesia

Neale D, Kremer A (2011) Forest tree genomics: Growing resources and applications. Nat Rev Genet 12:111-22. https://doi.org/10.1038/nrg2931

Nereu M, Silva JS, Deus E, Nunes M, Potts B (2019) The effect of management operations on the demography of *Eucalyptus globulus* seedlings. For Eco Man 453:117630. https://doi.org/10.1016/j.foreco.2019.117630

Nolan RH, Collins L, Leigh A, Ooi MKJ, Curran TJ, Fairman TA, Resco de Dios V, Bradstock R (2021) Limits to post-fire vegetation recovery under climate change. Plant Cell Environ 44(11):3471-3489. https://doi.org/10.1111/pce.14176

Nunes LJR, Meireles CIR, Pinto Gomes CJ, Almeida Ribeiro NMC (2019) Historical development of the Portuguese forest: The introduction of invasive species. Forests 10(11):974. https://doi:10.3390/f10110974

Pausas JP (2004) Changes in fire and climate in the Eastern Iberian Peninsula (Mediterranean Basin). Clim Change 63:337-350. https://doi:10.1023/B:CLIM.0000018508.94901.9c

Pausas J, Vallejo V (1999) The role of fire in European Mediterranean Ecosystems. In: Chuvieco E (ed) Remote sensing of large wildfires in the European Mediterranean basin. Springer-Verlag, pp 3-16

Pausas J, Keeley JE (2009) A burning story: the role of fire in the history of life. BioScience 59(7): 593-601. https://doi.org/10.1525/bio.2009.59.7.10

Payn T, Carnus JM, Freer-Smith P, Kimberley M, Kollert W, Liu S, Orazio C, Rodriguez L, Silva LN, Wingfield MJ (2015) Changes in planted forests and future global implications. For Eco Man 352:57-67. https://doi.org/10.1016/ j.foreco.2015.06.021

Popović Z, Bojović S, Marković M, Cerdà A (2021) Tree species flammability based on plant traits: A synthesis. Sci Total Environ 800:149625. https://doi.org/10.1016/j.scitotenv. 2021.149625

Potts B, Vaillancourt R, Jordan G, Dutkowski GW, Mckinnon G, Steane D, Volker P, Lopez G, Apiolaza LA, Li Y, Marques CMP, Borralho N, Silva J (2004) Exploration of the *Eucalyptus globulus* gene pool. In: Borralho N, Pereira JS, Marques C, Coutinho J, Madeira M, Tomé M (eds) *Eucalyptus* in a changing world. Proc. of IUFRO Conf., Aveiro 11-15 October 2004

Potts B, Hamilton M, Blackburn D (2011) Genetics of eucalypts: Traps and opportunities. In: Walker J (ed) Developing a eucalypt resource: Learning from Australia and elsewhere. Wood Technology Research Centre, University of Canterbury, New Zealand

Pyšek P, Jarošík V (2005) Residence time determines the distribution of alien plants. In: Inderjit (ed) Invasive plants: Ecological and agricultural aspects. Birkhäuser Verlag, Switzerland, pp 77-96. https://doi.org/10.1007/3-7643-7380-6_5

Pyšek P, Krivánek M, Jarosík V (2009) Planting intensity, residence time, and species traits determine invasion success of alien woody species. Ecology, 90(10):2734-2744. https://doi.org/10.1890/08-0857.1

Queirós L, Deus E, Silva JS, Vicente J, Ortiz L, Fernandes PM, Castro-Díez P (2020) Assessing the drivers and recruitment potential of *Eucalyptus globulus* in the Iberian Peninsula. For Eco Man 466:118147. https//doi.org/10.1016/j.foreco.2020.118147

Rego FC, Silva JS (2014) Wildfires and landscape dynamics in Portugal: A regional assessment and global implications. In: Azevedo JC, Perera AH, Pinto MA (eds) Forest landscapes and global change: Challenges for research and management, Springer. https//doi.org/10.1007/978-1-4939-0953-7_3

Rejmánek M, Richardson DM, Pyšek P (2005) Plant invasions and invasibility of plant communities. In: van der Maarel E, Franklin J (eds) Vegetation ecology. Blackwell Science, Oxford, UK, pp 332-355

Rejmánek M, Richardson DM (2011) The eucalypts. In: Simberloff D, Rejmánek M (eds) Encyclopedia of biological invasions. University of California Press, Berkeley and Los Angeles, pp 203-209

Rejmánek M, Richardson DM (2013) Trees and shrubs as invasive alien species - 2013 update of the global database. Divers Distrib 19:1093-1094. https://doi.org/10.1111/ddi.12075

Rezende GDSP, de Resende MDV, de Assis TF (2014) *Eucalyptus* Breeding for Clonal Forestry. In: Fenning T (ed) Challenges and opportunities for the World's Forests in the 21st Century. Forestry Sciences, vol 81. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7076-8_16

Richardson, DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: Concepts and definitions. Divers Distrib 6:93-107. https://doi.org/10.1046/j.1472-4642.2000.00083.x

Richardson DM, Rejmánek M (2011) Trees and shrubs as invasive alien species - A global review. Divers Distrib 17:788-809. https://doi.org/10.1111/j.1472-4642.2011. 00782.x

Ritter M, Yost J (2009) Diversity, reproduction, and potential for invasiveness of *Eucalyptus* in California. Madroño 56(3):155-167. https://doi.org/10.3120/0024-9637-56.3.155

Rix K, Gracie A, Potts B, Brown P, Gore P (2015) Genetic control of *Eucalyptus globulus* seed germination. Ann For Sci 72:457-467. https://doi.org/10.1007/s13595-014-0450-9

Rodrigues M, Camprubí AC, Balaguer-Romano R, Ruffault J, Fernandes P, Resco de Dios V (2023) Drivers and implications of the extreme 2022 wildfire season in Southwest Europe. Sci Total Environ 859(2):160320. https://doi.org/10.1016/j.scitotenv.2022.160320

Sánchez-Benítez A, García-Herrera R, Barriopedro D, Sousa PM, Trigo RM (2018) June 2017: The earliest European summer mega-heatwave of reanalysis period. Geophys Res Lett 45:1955-1962. https://doi.org/10.1002/2018GL077253

Sanz-Elorza M, Dana ED, Sobrino E (2001) Checklist of invasive alien plants in Spain (Iberian Peninsula and Balearic Islands). Lazaroa 22:121-131

Schulze SS, Fischer EC, Hamideh S, Mahmoud H (2020) Wildfire impacts on schools and hospitals following the 2018 California Camp Fire. Nat Hazards 104:901-925. https://doi.org/10.1007/s11069-020-04197-0

Sedjo TA (1999) Biotechnology and planted forests: assessment of potential and possibilities. Discussion Paper 00-06. Resources for the Future, Wash. D.C

Simberloff D (2009) The role of propagule pressure in biological invasions. Annu Rev Ecol Evol Syst 40:81-102. https://doi.org/10.1146/annurev.ecolsys.110308. 120304

Silva JS, Moreira F, Vaz P, Catry F, Godinho-Ferreira P (2009) Assessing the relative fire proneness of different forest types in Portugal. Plant Biosyst 143(3):597-608. https://doi.org/ 10.1080/11263500903233250

Silva JS, Vaz P, Moreira F, Catry F, Rego FC (2011) Wildfires as a major driver of landscape dynamics in three fire-prone areas of Portugal. Landsc Urban Plan 101(4):349-358. https://doi.org/10.1016/j.landurbplan.2011.03.001

Silva JS, dos Santos P, Sério A, Gomes F (2016) Effects of heat on dehiscence and germination in *Eucalyptus globulus* Labill. Int J Wildland Fire 25:478-483. https://doi.org/doi.org/10.1071/WF15078

Silva JS, Nereu M, Pinho S, Queirós L, Jesús C, Deus E (2021) Post-fire demography, growth and control of *Eucalyptus globulus* wildlings. Forests 12:156. https://doi.org/10.3390/f12020156

Souto XC, Bolaño JC, González L, Reigosa MJ (2001) Allelopathic effects of tree species in some soil microbial populations and herbaceous plants. Biol Plant 44(2):269-275. https://doi.org/10.1023/A:1010259627812

Stanturf J, Vance E, Fox T, Kirst M (2013) *Eucalyptus* beyond Its Native Range: Environmental Issues in Exotic Bioenergy Plantations. Int J For Res 463030. https://doi.org/10.1155/2013/463030

Steffen W, Crutzen PJ, McNeill JR (2007) The Anthropocene: Are humans now overwhelming the great forces of nature? Ambio 36(8):614-621. http://dx.doi.org/10.1579/0044-7447(2007)36[614:TAAHNO]2.0.CO;2

Stoneman GL (1994) Ecology and physiology of establishment of eucalypt seedlings from seed: A review. Aust For 57:11-29. https://doi.org/10.1080/00049158.1994.10676109

Tilman D, Clark M, Williams D, Kimmel K, Polasky S, Packer C (2017) Future threats to biodiversity and pathways to their prevention. Nature 546:73-81. https://doi.org/ 10.1038/ nature22900

Thompson I, Okabe K, Parrotta J, Brockerhoff E, Jactel H, Forrester D, Taki H (2014) Biodiversity and ecosystem services: Lessons from nature to improve management of planted forests for REDD-plus. Biodivers Conserv 23:2613-2635. https://doi.org/10.1007/ s10531-014-0736-0

Tomé M, Almeida MH, Barreiro S, Branco MR, Deus E, Pinto G, Silva JS, Soares P, Rodríguez-Soalleiro R (2021) Opportunities and challenges of *Eucalyptus* plantations in Europe: The Iberian Peninsula experience. Eur J For Res 140:489-510. https://doi.org/ 10.1007/s10342-021-01358-z

Turco M, Bedia J, Di Liberto F, Fiorucci P, von Hardenberg J, Koutsias N, Llasat, MC, Xystrakis, F, Provenzale, A (2016) Decreasing fires in Mediterranean Europe. PLoS ONE 11:e0150663. https://doi.org/10.1371/journal.pone.0150663

Turco M, Jerez S, Augusto S, Tarín-Carrasco P, Ratola N, Jiménez-Guerrero P, Trigo RM (2019) Climate drivers of the 2017 devastating fires in Portugal. Sci Rep 9:13886. https://10.1038/s41598-019-50281-2

Turnbull JW (1999) Eucalypt plantations. New For 17:37-52. http://doi.org/10.1023/A:1006524911242

Varghese M, Kamalakannan R, Harwood CE, Lindgren D, McDonald MW (2009) Changes in growth performance and fecundity of *Eucalyptus camaldulensis* and *E. tereticornis* during domestication in Southern India. Tree Genet. Genomes 5:629-640. https://doi.org/10.1007/s11295-009-0215-z

Vaz AS, Honrado JP, Lomba A (2019) Replacement of pine by eucalypt plantations: Effects on the diversity and structure of tree assemblages under land abandonment and implications for landscape management. Landsc Urban Plan 185:61-67. https://doi.org/10.1016/j.landurbplan.2019.01.009

Vilà M, Espinar JL, Hejda M, Hulme P, Jarošík V, Maron J, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: A meta-analysis of their effects on species, communities and ecosystems Ecol Lett 14:702-8. https://doi.org/10.1111/j.14610248.2011.01628.x

Wang J, Guan Y, Wu L, Guan X, Cai W, Huang J, Dong W, Zhang B (2021). Changing lengths of the four seasons by global warming. Geophys Res Lett 48(6): e2020GL091753. https://doi.org/10.1029/2020GL091753

Williamson M (2006) Explaining and predicting the success of invading species at different stages of invasion. Biol Invasions 8:1561-1568. doi.org/10.1007/s10530-005-5849-7

Wills SA, Williams CO, Duniway MC, Veenstra J, Seybold C, Presley D (2017). Human land-use and soil change. In: West L, Singer M, Hartemink A (eds) The soils of the USA. World Soils Book Series. Springer, Cham. https://doi.org/10.1007/978-3-319-41870-4_18

White TL (1987) A conceptual framework for tree improvement programs. New For 4:325-342. https://doi.org/10.1007/BF00031742

Whittock SP, Apiolaza LA, Kelly CM, Potts BM (2023) Genetic control of coppice and lignotuber development in *Eucalyptus globulus*. Aust J Bot 51:57-67. http://doi.org/10.1071/BT02049

Wolf K, DiTomaso J (2015) Management of blue gum *Eucalyptus* in California requires region-specific consideration. Calif Agric 70:39-47. https://doi.org/10.3733/ca.v070n01p39

Wu HX (2019) Benefits and risks of using clones in forestry - A review, Scand J For Res 34(5):352-359. https://doi.org/10.1080/02827581.2018.1487579

Zalesny R, Cunningham M, Hall R, Mirck J, Rockwood D, Stanturf J, Volk T (2011) Woody biomass from short rotation energy crops (Ch 2). In: Zhu JY (ed) Sustainable production of fuels, chemicals, and fibers from forest biomass. ACS symposium series. American Chemical Society, Washington, DC, pp 27-63. https://doi.org/10.1021/bk-2011-1067

Ziller SR, Dechoum MS, Zenni RD (2018) Predicting invasion risk of 16 species of eucalypts using a risk assessment protocol developed for Brazil. Austral Ecol 44(1):28-35. https://doi.org/10.1111/aec.12649

Chapter 2

MANAGEMENT AND FIRE, A CRITICAL COMBINATION FOR *EUCALYPTUS GLOBULUS* DISPERSAL

Anjos A, Fernandes P, Marques C, Borralho N, Valente C, Correia O, Máguas C, Chozas S (2021) Management and fire, a critical combination for *Eucalyptus globulus* dispersal. For Eco Man 490:91-102. https://doi.org/10.1016/j.foreco.2021.119086

Abstract

In a context of growing demands for wood and wood derived products, plantations of exotic tree species have globally increased. Fast growth and high productivity made *Eucalyptus* one of the most successful tree genera around the world. Nevertheless, this genus is often associated with negative ecological impacts on biodiversity and ecosystem functioning and the risk of expansion is considered a major threat. *Eucalyptus globulus* is the most planted tree species in Portugal, but common silvicultural measures, including periodic control of the understory vegetation, have traditionally limited natural regeneration. However, forest fires constitute a main driver of *E. globulus* dispersal and regeneration and, under the current climatic change scenario, the possible extension of the summer fire regime to previous months in spring and/or later months in autumn, may have a profound effect on *E. globulus* dispersal capacity. Moreover, isolated eucalypt trees, seed-trees, are often left uncut and many plantations are poorly managed potentially increasing the risk of *E. globulus* dispersal. To evaluate the impact of both management and fire event dates on *E. globulus* dispersal, we assessed the establishment of saplings beyond plantations and seed-tree boundaries in absence of fire and after 2017 June and October fires in managed and unmanaged conditions. Sapling survival was also analysed two years after fire. Our results point out that sapling establishment in our study area is not a major concern in the absence of fire. Also, our findings showed that *E. globulus* establishment is highly dependent on the time of the year a fire occurs and that pre-fire management practices constrain *E. globulus* dispersal. We also found that seed-trees are high seed dispersers after fire even in managed conditions, deserving great concern. Additionally, high sapling survival two years after October fire indicate that out of season fires might constitute an emerging issue regarding *E. globulus* expansion.

2.1 Introduction

Globally, the use of exotic tree species in planted forests is a major component of terrestrial land use in temperate and tropical regions and has been increasing in order to respond to growing demands for wood and wood derived products (Dodet and Collet 2012). Fast growth, high productivity, and product quality, ascribed to many exotic species, are the main reasons why they have been widely used (Turnbull 1999). *Eucalyptus* is one of the most successful tree genera around the world. Being native from Australia it is nowadays rated the second most cultivated tree worldwide (Rejmánek and Richardson 2013). Despite their economic and social benefits, exotic tree species such as eucalypts are often associated with negative ecological impacts on biodiversity and ecosystem functioning (Richardson 1998; Hartley 2002), including the risk of expansion to surrounding areas where they may outcompete native species (Richardson and Rejmánek 2011).

In Portugal, *E. globulus* Labill. (Tasmanian blue gum), which was introduced in the middle of the 19th century (Alves *et al.* 2007), occupies nowadays ca. 845 000 ha (ICNF 2019) and is one of the main forest species in the country. Most *E. globulus* stands in Portugal have been planted intentionally and are managed for pulpwood production in rotation cycles of 10-12 years, usually followed by a second or third coppice rotation (Soares *et al.* 2007). Recommended silvicultural measures include periodic control of the understory vegetation to reduce fire hazard and weed competition for water and nutrients. As a result, any putative regeneration of *E. globulus* is likely to be destroyed by vegetation control measures (Larcombe *et al.* 2013; Fernandes *et al.* 2016a). Nonetheless, isolated *E. globulus* trees are often left uncut and many plantations are poorly managed, resulting in the presence of old trees, often large and with high canopies with great reproductive capacity, potentially increasing the risk of *E. globulus* dispersal. In particular, these dispersed trees, named as seed-trees, are potential seed-sources (Adams *et al.* 1994) whose presence may have critical implications for *E. globulus* natural regeneration dynamics.

Eucalyptus globulus mature seeds are held in the canopy, inside the capsules. They remain there for months or even a few years (Lamont *et al.* 1991). Seeds will eventually be released and dispersed, as branches dry out and fall. This process is more frequent during autumn and winter (Calviño-Cancela and Rubido-Bará 2013), or soon after a fire (Gill 1997).

Given the wide range of environmental conditions and disturbances in which it has been established (Kirkpatrick 1975) there have been concerns about *E. globulus* ability to regenerate and establish wildlings beyond plantations edges. These concerns resulted in several studies and Weed Risk Assessments have recommended *E. globulus* to be considered an invasive species in Europe, the Americas, New Zealand and the Pacific and Indian Ocean islands (e.g. Sanz-Elorza *et al.* 2001; Gassó *et al.* 2010; Gordon *et al.* 2012; Rejmánek and Richardson 2013; Marchante *et al.* 2014). Nonetheless, other studies ranked *E. globulus* with moderate risk of invasion, including in well managed plantations (Larcombe *et al.* 2013; Fernandes *et al.* 2016a; Ziller *et al.* 2018). In fact, Rejmánek and Richardson (2011) concluded that eucalypts have a low invasiveness potential compared with most other commercially important tree species, due to limited seed dispersal, high seedling mortality, and lack of compatible ectomycorrhizal fungi. Fernandes *et al.* (2016a) reported that *E. globulus* seedlings are not tolerant to drought stress, with high mortality immediately after emergence, despite the fact that once seedlings overcome the first two months, mortality rate decreases, as also suggested by Calviño-Cancela and Rubido-Bará (2013).

A key factor that impacts the risk of dispersal and regeneration are forest fires (Fernandes *et al.* 2016b). This is particularly critical in fire prone regions of Mediterranean-type climate with long dry summers, such as Portugal (Salis *et al.* 2014). Under the current climatic change scenario, the frequency of out of season fires is likely to increase (Turco *et al*. 2019), and may change the known pattern of this species expansion.

In Portugal, 2017 was a particularly dry and warm year, which resulted in several large wildfire events from June (early in the season) to mid-October (late in the season). As a consequence of the June fires ca. 52 000 hectares (ha) burned, while the October fires devastated an even larger area (ca. 190 000 ha) (ICNF 2017). Fire events are a critical factor in stimulating *E. globulus* regeneration, through dorment bud’s sprouting and seedling recruitment (Silva and Marchante 2012; Larcombe *et al.* 2013; Águas *et al.* 2014, Calviño-Cancela *et al.* 2018), but seasonal differences have not been studied in detail. High fire risk and a longer fire season raise concerns about more extensive naturalization events and an increase in potential invasive behaviours.

The present study looks at the effects of fire, including out of season events, and the compounding impact of forest management, along with the presence of seed-trees, on the success of *E. globulus* recruitment. In particular, we hypothesize that natural regeneration and potential expansion of *E. globulus* outside plantations and beyond the isolated trees (seed-trees) are affected by fire and management practices. However, we expect that high sapling mortality will limit *E. globulus* spread. In this context, the main objective of this study is to evaluate the impact of the 2017 June and October forest fires and previous plantation management on the natural regeneration and expansion capacity of *E. globulus* saplings. In order to achieve this, we evaluated the risk of *E. globulus* expansion by i) assessing the establishment of *E. globulus* saplings outside plantations edges and in the surrounding areas of isolated seed-trees in the absence of fire and after two distinct fire events, ii) analysing the role of vegetation management actions in establishment outside plantation boundaries and area surrounding seed-trees, to ultimately mitigate the potential invasive behaviour and finally iii) assessing the survival rate of saplings two years after fire occurrence. We expect this information will inform management decisions aimed at mitigating the risk of *E. globulus* invasion.

2.2 Material and Methods

2.2.1 Study area

This study was carried out in Northern and Central Portugal, regions of higher productivity for *E. globulus* (Alves *et al*. 2007), in sites affected by the fire events that occurred on 17th June 2017 (Castanheira de Pêra (CDP) and Pedrógão Grande (PG)) and on 15th October 2017 (Castelo de Paiva (CSP), Mira (M), São Pedro de Alva (SPA) and Pampilhosa da Serra (PS)) (Fig. 2.1a). Climate in the study area is temperate with dry and mild summer (Csb), according to Köppen climate classification (IPMA 2021), with mean annual temperatures ranging from 10.5 to 14.6 °C and total precipitation ranging from 952 to 1440 mm (IPMA 2021). Elevation varies from 15 to 735 m. This study area experienced a very dry winter and spring in 2017 (Fig. 2.1e and f). Site characterization is included in Supplementary Table S2.1. Within the area affected by the June fire, three managed and three unmanaged *E. globulus* plantations were selected. In the October fire-affected areas, seven managed and five unmanaged *E. globulus* plantations were studied. Fourteen and 28 isolated seed-trees, affected by the June and the October fires, respectively, and 42 unburnt isolated seed-trees were also studied. Simultaneously, 18 unburnt unmanaged plantations located close to the selected burnt plantations were sampled. Moreover, data from a previous work with unburnt managed plantations (Fernandes *et al*. 2016a) was included in this study, where the same sampling design was applied and selecting only data from the same climatic region. After fire, plantations were classified as managed when no large trunks of shrubs and other tree species were observed (suggesting that an effective understory vegetation control was carried out) and in the case of coppice stands when the number of stems per stump were less or equal to three (which would indicate that appropriate thinning of the stand was carried out). In order to classify the type of stand management around seed-trees, surrounding areas were considered. Likewise, these areas were considered as managed if no large trunks of burnt trees or shrubs in nearby forest or shrublands were observed.

Sampling procedures required meeting several stringent criteria that highly restricted plantation and seed-tree selection. First, in order to ensure that the *E. globulus* saplings recorded belonged to individuals from the studied plantation, plantations were selected

(i) without any *E. globulus* individuals in their adjacent areas; (ii) located at least 70 m apart from another *E. globulus* plantation and (iii) with no seed-trees nearby. The presence of seed capsules on the *E. globulus* trees was verified, to ensure the potential for natural regeneration. The size of the plantations, with preference for larger areas, and good accessibility were additional conditions for plantation selection. Finally, sampling for seed-trees implied the selection of trees (i) isolated from other seed-trees at least 70 m; (ii) with at least 35 cm of DBH (Diameter at Breast Height) and; (iii) with a completely burnt canopy.

Both plantations and seed-trees were classified according to Type (plantations or seed-trees), Fire occurrence (No fire and June or October fire) and Management (managed or unmanaged) (Fig. 2.1b, c and d; Tab. 2.1).

2.2.2 Field sampling

2.2.2.1 Assessing *E. globulus* dispersal and natural regeneration

Sampling was performed from September to December 2018, about one year after the fires. Data were collected through transects from *E. globulus* plantation edge to adjacent areas occupied by other habitats (Fig. 2.1g). In each plantation, at least three transects (plantation transects) were established 15 m apart, perpendicular to the plantation's boundary edge, and each transect was divided in 3x3 m plots. The first plot was established at 0 or 3 m outside the plantation and then plots were sampled alternately (adapted from Callaham *et al*. 2013). Number and cover of *E. globulus* saplings were quantified in each sampled plot. *Eucalyptus globulus* saplings were distinguished from coppice or planted individuals, through its position and size relatively to the planted trees (unkempt plantation lines) that were mostly already resprouting.

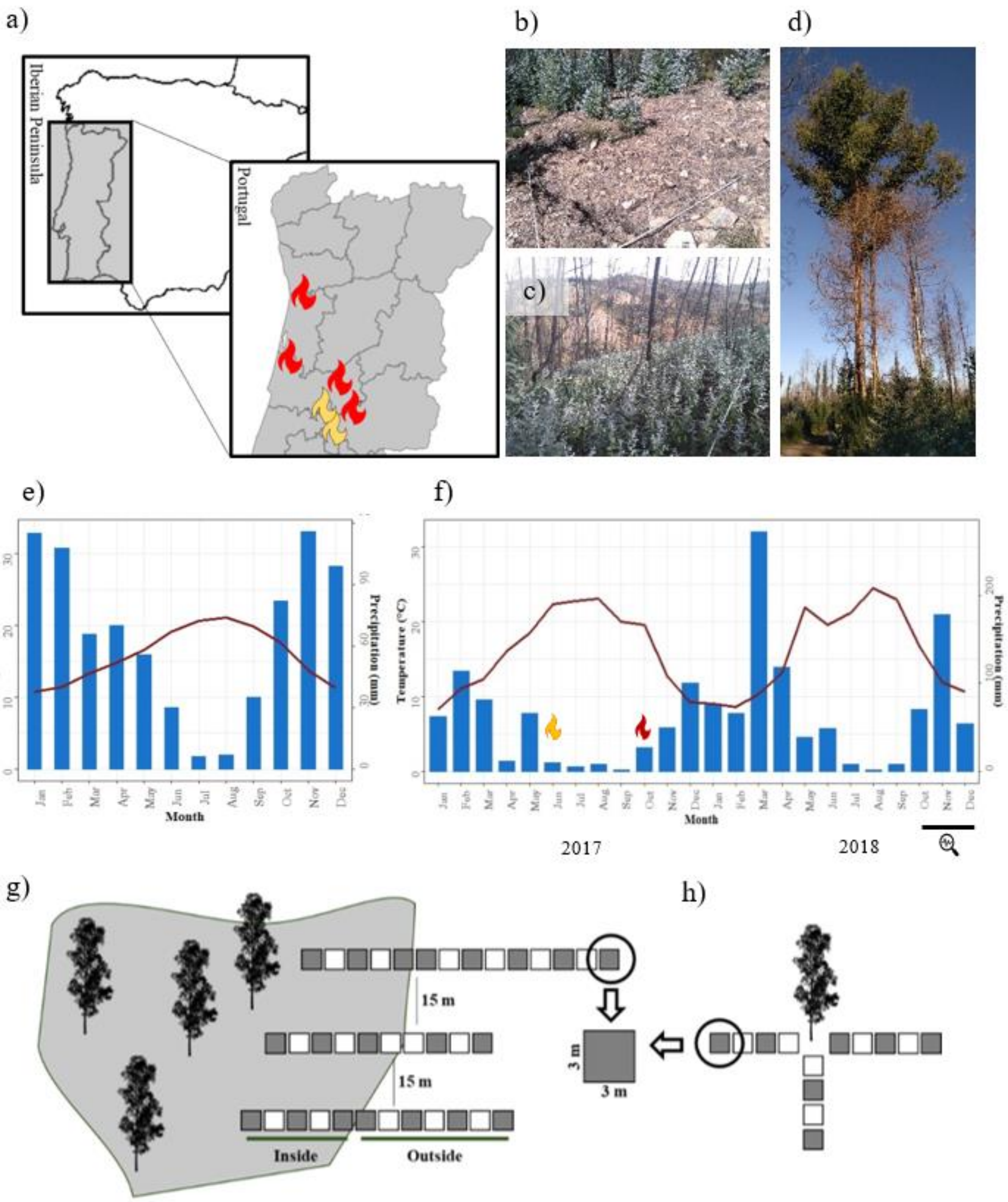


Figure 2.1 Location of the study regions in Portugal: a) study sites) affected by June fire are highlighted in yellow (Castanheira de Pêra (CDP) and Pedrógão Grande (PG)), while study sites affected by October fire are highlighted in red (Castelo de Paiva (CSP), Mira (M), São Pedro de Alva (SPA) and Pampilhosa da Serra (PS)); b) burnt managed *E. globulus* plantation; c) burnt unmanaged *E. globulus* plantation; d) burnt *E. globulus* seed-tree; e) monthly average temperature [ºC] (line) and monthly average precipitation [mm] (bars) in study areas from 1982 to 2012 (Climate-date.org) and f) from January 2017 to December 2018, with fire events and October to December 2018 data collection highlighted. Schematic diagrams of transects design in plantation (g) and seed-trees (h): transects were established with 3×3 m plots. Only grey plots were sampled. Transects were oriented perpendicular to the plantation boundary edge or to the seed-tree.

Transect sampling finished when two consecutive plots had no *E. globulus* saplings, marking the limit of the *E. globulus* expansion. Within the plantation, three alternate plots were always sampled (starting at the symmetrical distance of the outside transect, i.e., if the first outside plot was sampled at 3 m, the same occurs to the inside plot, marked as -3 m (Fig. 2.1g).

Similarly, to assess *E. globulus* natural regeneration around seed-trees, at least two perpendicular transects (seed-tree transects) were sampled in each seed-tree with a maximum of four transects per tree (Fig. 2.1h). In what concerns seed-trees, management type was classified at transect level, mostly due to location near roads and/or adjacent habitats. Sampling finished when two consecutive plots had no *E. globulus* saplings. This means that, in both cases, there were always at least two plots to the outside of the plantation and in the surrounding areas of seed-trees. The total number of transects sampled is summarized in Table 2.1.

Table 2.1 Total number of transects sampled within the study area, including plantation and seed-tree transects, both managed and unmanaged and organized by Fire Occurrence (No Fire, June Fire and October Fire).

	No fire		**June Fire**		**October Fire**	
	Managed	**Unmanaged**	**Managed**	**Unmanaged**	**Managed**	**Unmanaged**
Plantation	277	54	10	13	30	21
Seed-tree	56	86	27	20	39	45

Due to the high density of saplings along the seed-tree transects and the difficulty to count all of them, the possibility of using sapling cover instead was evaluated. The relationship between the total number and the area covered by the *E. globulus* saplings was examined through a Spearman correlation (Supplementary Figure S2.2) and, as expected, Spearman r value (r=0.88) confirmed that both parameters were highly correlated and consequently we proceeded to use sapling cover to characterize the incidence of natural regeneration.

2.2.2.2 Assessing *E. globulus* sapling survival

Considering only *E. globulus* burnt plantations, permanent plots with 1 m radius were set, in which *E. globulus* sapling number was assessed. These permanent plots were included inside 3x3 m plots sampled. To evaluate sapling survival, these permanent plots were also monitored in October 2019, about two years after the fires, and the remaining saplings were recounted.

2.2.3 Data analysis

Eucalyptus globulus dispersal curves focused on fire events were constructed by plotting sapling mean cover for each distance from inside and outside the plantation edge and from each seed-tree. Then, a multivariate local polynomial regression estimator (Loess) fitting was applied. Loess is a non-parametric method that fits a quadratic surface by weighted least squares (Cleveland and Devlin 1988). To assess the main factors affecting dispersal distances in *E. globulus* after fire, we used a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution to account for unbalanced design and overdispersion associated with the high number of zeros in the data. Fire occurrence (only "June fire" or "October fire"), Management and Type were included in the model as fixed factors and the variable Site as random effect (Tab. 2.2). Maximum distance reached by saplings was defined as the dependent variable.

Table 2.2 Description of explanatory variables.

Variable	Data description
Type	Plantation or isolated seed-tree
Fire occurrence	No fire, June or October fire event
Management	Managed - trunks *per* tree were less or equal to three and if large trunks of burnt shrubs or trees were not observed Unmanaged - trunks *per* tree were higher than three and if large trunks of burnt shrubs or trees were observed

Similarly, to assess the factors that influenced *E. globulus* natural regeneration, sapling cover was also modelled performing a GLMM with a negative binomial distribution and using Fire occurrence, Management and Type as fixed factors and Site as random factor. In order to guarantee the same number of sampled plots in each transect (i.e., two plots, see Field sampling), only the plots located within the first 15 m outside of the plantations and around seed-trees were considered. Finally, to determine which groups were significantly different, multiple pairwise comparisons of estimated marginal means were calculated, using the *emmeans* function. Bonferroni correction method was applied to adjust *p* values to multiple comparisons.

Sapling survival was evaluated through the comparison between the number of saplings counted one year (2018) and two years (2019) after June and October fires, in sampled plantations using Wilcoxon tests.

Data analysis was performed using packages *stats*, *glmmADMB* and *emmeans* in R studio software (v.3.6.1).

2.3 Results

2.3.1 Fire date and management effects in *E. globulus* dispersal

Sapling cover and distance to both plantation edges and seed-trees showed a clear space-dependent relationship. The fit between sapling cover and distance to plantation edge indicated that, although there were no statistically significant differences (data not shown), highest cover of saplings always occurred inside plantations (from -15 to 0 m), decreasing to the outside. This pattern was also observed along the isolated seed-tree transects, with the maximum cover of *E. globulus* saplings observed close to the seed-trees (from 0 to 15 m) and the cover values decreasing with increasing distance from these trees.

GLMM results indicated that Seed-tree, October fire and Unmanaged condition are significantly increasing the distance at which *E. globulus* saplings occur (Tab. 2.3). For both plantations and seed-tree transects sapling cover was significantly higher after the October fire than that observed after the June fire (Fig. 2.2).

When comparing unmanaged and managed conditions, in addition to a greater sapling cover, in the first, distances reached by *E. globulus* saplings were higher in unmanaged conditions for both plantations and seed-trees. Also, after the October fire, saplings were found at greater distances (45 and 75 m in plantation and seed-tree transects, respectively), while in plantations and seed-trees burnt in June, saplings reached 15 and 30 m, respectively. Saplings were observed to a maximum distance of 99 m away from seed-trees and 69 m from unmanaged plantations edge after the October fire, but at very low frequencies (with only one sapling observed in each case).

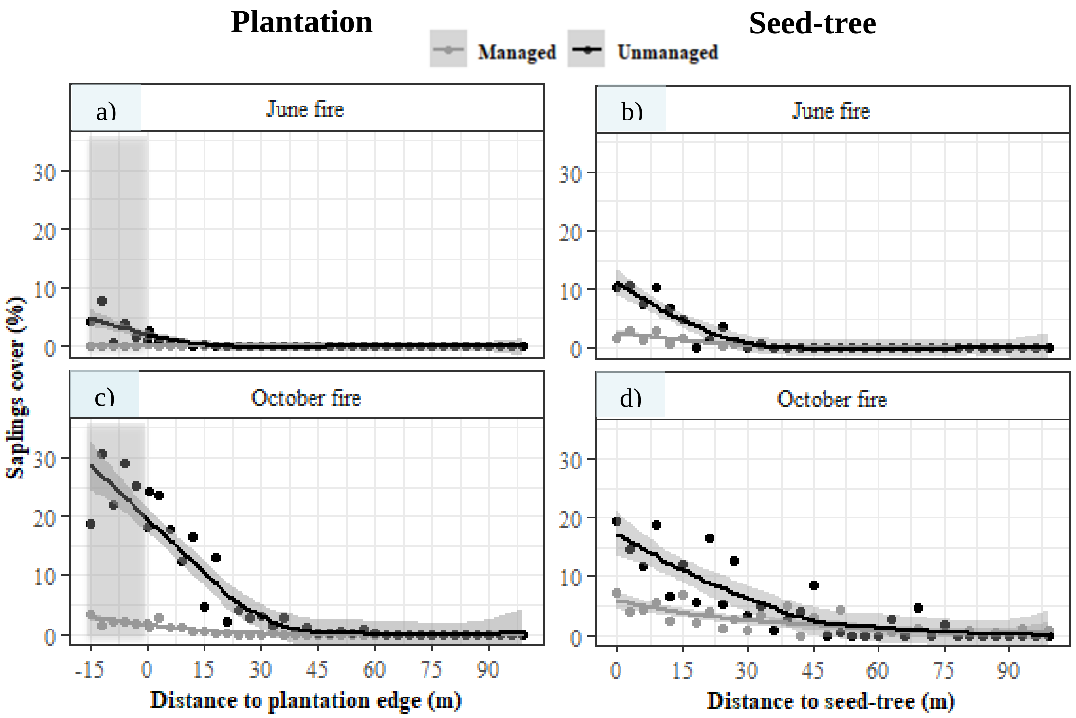


Figure 2.2 Dispersal curves of *E. globulus* saplings after the June and October 2017 fires, based on average sapling cover (%) to the edge of managed and unmanaged *E. globulus* plantation (a and c) and managed and unmanaged surrounding areas of *E. globulus* seed-trees (b and d) using Local Polynomial Regression Fitting (Loess). Shaded area represents 95% confidence intervals. Negative distances (highlighted in grey) represent the inside of *E. globulus* plantations. The distance of 0 m represents plantation edge and seed-tree location.

Table 2.3 Generalized linear mixed model (GLMM) using *Type* (Plantation or Isolated Seed-tree), *Fire date* (June fire and October fire) and *Management* (Managed or Unmanaged) to model maximum distance reached by *E. globulus* saplings. Coefficients of the model, standard errors, the z statistic and the associated probabilities: **: ≤ 0.01; *: < 0.05.

	Estimate	**Std. Error**	**z value**	**Pr(> |z|)**
(Intercept)	-2.306	1.167	-1.98	**0.048 ***
October fire	2.687	1.237	2.17	**0.029 ***
Seed tree	3.259	1.151	2.83	**0.005 ****
Unmanaged	2.626	1.209	2.17	**0.029 ***
October fire * Seed tree	-2.139	1.223	-1.75	0.080
October fire *Unmanaged	-0.091	1.311	-0.07	0.945
Seed tree * Unmanaged	-1.316	1.293	-1.02	0.309
October fire * Seed tree * Unmanaged	-0.169	1.436	-0.12	0.906

2.3.2 *Eucalyptus globulus* sapling establishment and survival

Considering *E. globulus* establishment, GLMM results showed that all studied factors were influencing sapling cover in our study area (Tab. 2.4).

Table 2.4 Generalized linear mixed model (GLMM) using *Type* (Plantation or Isolated Seed-trees), *Fire occurrence* (No fire, June fire and October fire) and *Management* (Managed or Unmanaged) to model *E. globulus* sapling cover. Coefficients of the model, standard errors, the z statistic and the associated probabilities: **: ≤0.01; *: < 0.05.

	Estimate	**Std. Error**	**z value**	**Pr(> |z|)**
(Intercept)	-2.490	1.229	-2.03	**0.043 ***
No fire	2.226	1.223	1.82	0.069
October fire	3.072	1.296	2.37	**0.018 ***
Seed tree	3.337	1.283	2.60	**0.009 ****
Unmanaged	2.742	1.369	2.00	**0.045 ***
No fire * Seed tree	-5.565	1.412	-3.94	**$8.1e^{-5}$ ****
October fire * Seed tree	-2.183	1.393	-1.57	0.117
No fire * Unmanaged	-6.466	1.725	-3.75	**$1.8e^{-4}$ ****
October fire *Unmanaged	-0.176	1.519	-0.12	0.908
Seed tree * Unmanaged	-1.452	1.510	-0.96	0.336
No fire * Seed tree * Unmanaged	4.947	1.988	2.49	**0.013 ***
October fire * Seed tree * Unmanaged	-0.122	1.711	-0.07	0.943

The absence of fire revealed the lowest sapling cover (around 0%) when compared to the occurrence of fire, both in plantations and seed-trees, with no significant statistical differences observed also between managed and unmanaged regimes (Fig. 2.3).

After the June fire, the highest mean sapling cover was observed under unmanaged seed-trees (9.2±2.5%), while managed and unmanaged plantations and managed seed-trees showed low sapling cover (0.1±0.1%, 1.4±0.8% and 2.4±0.7%, respectively) with no statistically significant differences. On the other hand, the October fire showed heterogeneous results, with low sapling cover in managed plantations (1.5±0.5%), followed by managed adjacent seed-trees areas (6.5±1.8%) while sapling cover had the highest value in both unmanaged plantations and unmanaged adjacent areas to seed-trees (19.4±4.4% and 13.8±2.8%, respectively) (Fig. 2.3). Comparing *E. globulus* sapling cover from plantations and seed-trees, higher sapling cover under seed-trees was always observed, except when comparing with unmanaged plantations, after the October fire.

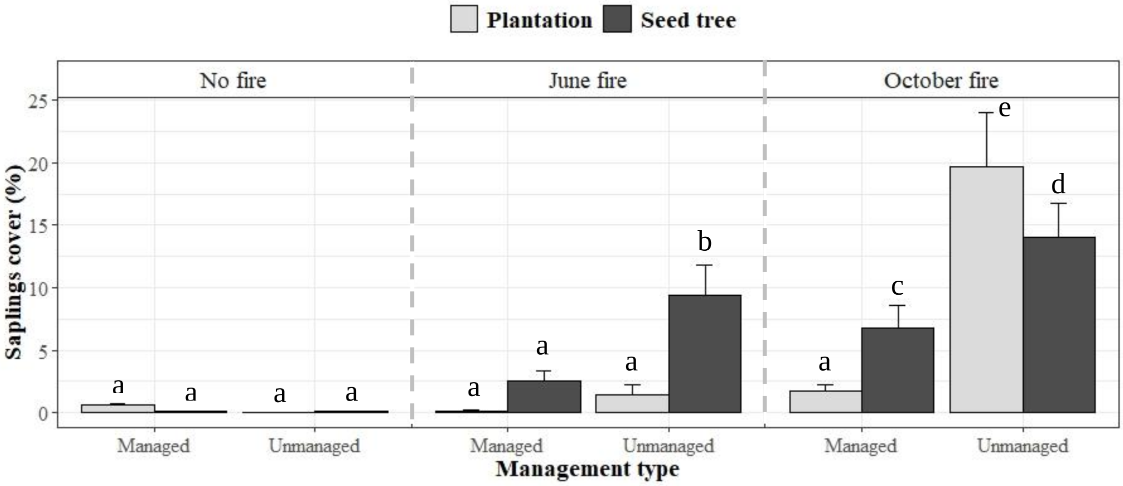


Figure 2.3 *Eucalyptus globulus* sapling cover (%; mean ± SE) for managed and unmanaged *E. globulus* plantations and managed and unmanaged surrounding areas of seed-trees in absence of fire (No Fire) and after June and October 2017 fires. Letters indicate significant differences based on multiple pairwise comparisons of estimated marginal means with Bonferroni adjustment.

Furthermore, Wilcoxon tests showed statistically significant differences between the number of *E. globulus* sapling counted in 2018 and recounted in 2019, except in managed plantations affected by the June fire (Fig. 2.4).

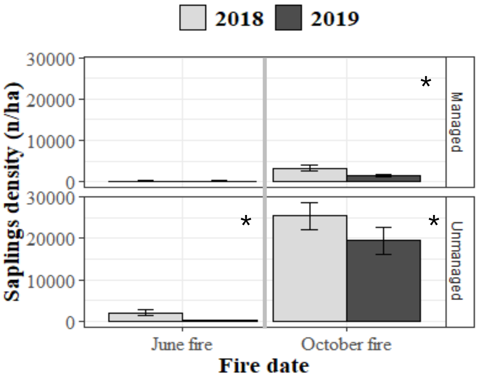


Figure 2.4 Wilcoxon tests comparing *E. globulus* sapling density (n/ha; mean ±SE) assessed in 2018 and 2019 for managed and unmanaged *E. globulus* plantations affected by the June and October 2017 fires. Asterisks represent significant differences (* $p<0.05$).

In managed plantations affected by the June fire, sapling number remained the same (128 saplings/ha) while in unmanaged plantations, a decrease was observed (2008 to 175 saplings/ha). Managed and unmanaged plantations burnt in October followed the same trend (a decreased from 3255 to 1498 and from 25413 to 19414 saplings/ha, respectively, from 2018 to 2019). It is important to highlight that, despite the low number of saplings, survival was higher in managed plantations after the June fire (100% of survival). Notwithstanding, unmanaged plantations affected by the June fire revealed 25% of sapling survival. After the October fire, both managed and unmanaged plantations showed almost 75% of sapling survival (data not shown).

2.4 Discussion

Our study found that the time of the fire event (naturally associated with after-fire weather conditions) is determinant for the success of *E. globulus* natural regeneration. In addition, management before fire is crucial to reduce the dispersal and degree of sapling cover.

It was also observed that the existence of seed-trees greatly contributes to *E. globulus* expansion. Moreover, our results indicate that spring and autumn fire events have an additional impact on sapling establishment. After the June fire, sapling cover was always lower than that found after the October fire. Nevertheless, differences were only significant for unmanaged plantations, underlying the importance of the synergistic effects between these factors. Most seedlings germination occurred immediately after fire (Pryor 1976), when bare soil and favourable weather conditions were available, which is more likely to occur after an autumn fire (October fire) which usually precedes wet weather, than after a spring fire (June fire) which is usually followed by dry summer conditions.

Previous studies with spring sowing registered high mortality of *E. globulus* seedlings, associated with high temperatures and drought (Fernandes *et al.* 2017). Furthermore, dos Santos *et al.* (2015) showed that seed release from capsules held in scorched branches could last eight weeks and Nereu *et al.* (2019) suggested that ca. 70% of seedlings germinated within the first month after sowing. Taking into consideration this information and the fact that 2017 summer was extremely hot and dry, we suggest that the low sapling cover found after the June fire could be associated with high mortality due to dry conditions. Consequently, saplings found after the June fire were therefore either able to survive the summer drought or germinated in the rainy season as suggested by Silva *et al.* (2021), which mentions recruitment as a *continuum*. Thus, regardless of the existence of management actions, the June fire has low *E. globulus* natural regeneration.

On the other hand, unmanaged areas surrounding seed-trees had a higher sapling cover. The absence of management and clearing actions lead to a conspicuous capsule accumulation on the soil (ca. up to 10 cm depth, field observations). During a fire only the capsules located in the upper soil layer burn, while the ones of the lower layers remain intact, protecting seeds from the heat damage (dos Santos *et al.* 2015), which may enable later germination. Additionally, sprouting from seed-trees up in the canopy (Fig. 1d) may provide moderate shade and decrease high soil temperatures, creating more favourable conditions that facilitate seedling recruitment and survival, contributing to the higher sapling cover observed.

Conversely, after the October fire, due to the subsequent rainy season, weather conditions were favourable to seedling establishment, resulting in high sapling cover one year after fire. However, managed plantations showed low sapling establishment. It is known that *E. globulus* reaches sexual maturity at three to four years (Jordan *et al.* 1999), but in dense plantations seed production is suppressed and does not usually occur until trees are at least seven years old (Kirkpatrick 1975). In fact, rotation cycles of 10-12 years reduce seed accumulation because trees are harvested before reaching their full potential of seed production, which could explain the low sapling cover within managed plantations. Also, capsules accumulation in the canopy is lower than in unmanaged plantations, where trees are left uncut for many years, accumulating capsules produced in different years, increasing their reproductive capacity (Barbour *et al.* 2008). Additionally, studies are needed to compare capsules accumulation in the canopy and in the soil as well as seed accounting and viability in trees with different ages, including seed-trees.

Similarly to other studies performed in Portugal (Fernandes *et al.* 2016a; Águas *et al.* 2017), we confirmed that in the absence of fire, *E. globulus* sapling cover in surrounding areas to both *E. globulus* plantations and seed-trees is extremely low. *Eucalyptus* seeds require wet and bare soil to germinate (Rejmánek and Richardson 2011) and in the absence of fire, areas outside plantations are dominated by vegetation that compete (Garau *et al.* 2009) and shade the soil surface. Additionally, the accumulation of litter on the soil hinders *E. globulus* seedlings emergence (Mount 1964; Águas *et al.* 2017). Also, litter prevents capsules and seeds from falling directly on the soil surface, decreasing the emergence rate (Calviño-Cancela *et al.* 2018). These effects, combined with the fact that seed recruitment is positively related to disturbance (Fernandes *et al.* 2018), may explain the lack of *E. globulus* germination in the absence of fire and the increase of sapling cover found in plantations affected by fire. After a fire, seeds have favourable environmental conditions for their establishment, namely greater light and nutrient availability as a result of the combustion of vegetation and litter (Calviño-Cancela *et al.* 2018).

In this study, seed-trees were considered as a key propagule source, due to their age and seed production potential. Isolated seed-trees showed higher sapling cover, except when comparing with unmanaged plantations affected by the October fire. This may be due to a much higher capsule production capacity (field observations) and lack of competition from coppice or sprouting from pre-existing trees in plantations (Potts 1986). Higher sapling cover in unmanaged plantations after the October fire compared to seed-trees could be due to the higher seed production ascribed to the higher number of trees present in plantations.

Regarding sapling establishment distances, for all the studied transects, a clear decrease was observed from both *E. globulus* plantations edges and *E. globulus* seed-trees, until around 45 m. Maximum saplings distances were 69 m away from plantations (similar to Deus *et al.* 2019) and 99 m from seed-trees, both observed in unmanaged situations after the October fire. Greatest distances reached by saplings from seed-trees are probably due to the higher height of these trees (ca. 17 m comparing with 10 m in plantations), since it is known that *E. globulus* seeds do not have an effective wind dispersal mechanism (Kirkpatrick 1977). Expected distances are reported to follow approximately twice the tree’s height (Cremer 1977). Moreover, the greatest cover of saplings was always found within the plantations and along the first 15 m outside the plantations, corresponding largely with results observed by other authors (Calviño-Cancela and Rubido-Bará 2013; Larcombe *et al*. 2013; Fernandes *et al.* 2016a; Águas *et al.* 2017; Deus *et al.* 2019), reflecting the limited seed dispersal ability of *E. globulus*. Further studies could be performed through geographic modelling of *E. globulus* expansion, to better understand its behaviour and dispersal risk, as suggested in the Global Guidelines concerning non-native trees use (Brundu *et al.* 2020).

Ashton and Chinner (1999) described that there is an antagonist set of conditions between germination and establishment, since for seedlings emergence bare soil is crucial, while sapling establishment requires shadowed conditions, associated with water availability. Hence, saplings assessed one year after fire overcame a high mortality period (Calviño-Cancela and Rubido-Bará 2013) and found favourable conditions for establishment. It is important to add that, one-third of *E. globulus* plantations were managed after fire and some two years saplings were eliminated by those practices, as reported by Águas *et al.* (2014). Moreover, it was observed a high mortality in permanent plots, maybe due to interspecific competition like herbaceous and shrubs (Fernandes *et al.* 2018; Deus *et al.* 2019) and also because one-year old saplings are more sensitive (Garau *et al.* 2009). Most of the saplings recounted two years after fire, were more than 1.5 m in height and, as suggested by

Adams *et al*. (2003), were able to overcome potential competition from other species. However, at high *E. globulus* sapling densities reduction in sapling number is expected, as a consequence of the increasing intra-specific competition.

Other studies performed in burnt areas in Portugal reported 8 800 *E. globulus* plants/ha five years after fire in abandoned plantations (Silva and Marchante 2012 and references within) and 4 800 plants/ha seven years after fire (Águas *et al.* 2014). Nevertheless, these studies are likely to be site and year dependent. We found that unmanaged plantations burnt in October 2017 showed a significantly higher sapling density (ca. 20 000 saplings/ha, two years after fire). This data represents the worst-case scenario: an autumn fire, followed by rain and combined with the absence of plantation management. The synergetic effect observed between fire and poor management highlights the importance of forest management practices in the control of natural regeneration and expansion of *E. globulus*. Along with *E. globulus* plantations, we emphasize the importance of isolated seed-trees across the Portuguese landscape in the establishment and expansion of this species. We underline the importance of considering it as an additional factor when planning control actions to prevent *E. globulus* expansion beyond plantations.

Conclusions

This study clarified the conditions in which *E. globulus* could represent a risk of significant dispersal to areas surrounding plantations or isolated seed-trees after a fire. Our findings indicate that *E. globulus* establishment is highly dependent on both the time of the year a fire occurs and the management practices implemented prior to fire. In the absence of fire, even in unmanaged plantations, sapling establishment does not seem to be a major concern in our study area (mainly Central Portugal), especially when fires occur early in the season followed by several dry months during summer. In this context, adequate management seems to be an essential measure to prevent *E. globulus* natural dispersal inside or around the borders of plantations once regular harvesting will decrease capsule production and its accumulation, associated with younger age of trees. Furthermore, even when autumn fire events occur, just before the rainy season, *E. globulus* sapling establishment in well managed plantations is very low. On the other hand, we found a significant increase in sapling cover in both unmanaged plantations and isolated seed-trees after the October fire. Therefore, it is critical to consider that, under a warmer weather, changes in frequency, intensity and seasonality of fires are expected, influencing *E. globulus* dispersal behaviour. In this context, adequate management seems to be an essential measure to prevent *E. globulus* establishment inside or around the borders of plantations. Furthermore, we found that isolated seed-trees seem to be an overall effective seed disperser after the advent of a fire, regardless of previous management actions.

Future studies should assess whether other specific local variables along with out of season fires occurrence could further influence sapling establishment and survival. Once it is known that these events are becoming more frequent it is crucial to fully understand the potential invasive behaviour of the species, especially considering autumn fires that are followed by rainy season. By increasing our knowledge about *E. globulus* expansion risks, we will contribute to improve best practices in plantation management of this species in Portugal.

References

Adams DL, Hodges JD, Loftis DL, Long JN, Seymour RS, Helms, JA (1994) Silviculture terminology with appendix of draft ecosystem management terms. Silviculture Instructors Subgroup of the Silviculture Working Group of the Society of American Foresters

Adams P, Beadle C, Mendham N, Smethurst PJ (2003) The impact of timing and duration of grass control on growth of a young *Eucalyptus globulus* Labill. plantation. New For 26:147-165. https://doi.org/10.1023/A:1024490707175

Águas A, Ferreira A, Maia P, Fernandes PM, Roxo L, Keizer J, Silva JS, Rego FC, Moreira F (2014) Natural establishment of *Eucalyptus globulus* Labill. in burnt stands in Portugal. For Eco Man 323:47-56. https://doi.org/10.1016/j.foreco. 2014.03.012

Águas A, Incerti G, Saracino A, Lanzotti V, Silva JS, Rego FC, Mazzoleni S, Bonanomi G (2017 Fire effects on litter chemistry and early development of *Eucalyptus globulus*. Plant Soil 422 (1-2):495-514. https://doi.org/10.1007/s11104-017-3419-2

Alves AM, Pereira JS, Silva JN (2007) O eucaliptal em Portugal - Impactes ambientais e investigação científica. ISAPress, Lisbon

Ashton DH, Chinner JH (1999) Problems of regeneration of the mature *Eucalyptus regnans* F. Muell, (The Big Ash) forest, in the absence of fire at Wallaby Creek, Victoria, Australia. Aust For 62(3):265-280. https://doi.org/10.1080/00049158.1999.10674791

Barbour RC, Otahal Y, Vaillancourt RE, Potts BM (2008) Assessing the risk of pollen-mediated gene flow from exotic *Eucalyptus globulus* plantations into native eucalypt populations of Australia Biol Conserv 141:896-907

Brundu G, Pauchard A, Pyšek P, Pergl J, Bindewald AM, Brunori A, Canavan S, Campagnaro T, Celesti-Grapow L, Dechoum M de S, Dufour-Dror JM, Essl F, Flory SL, Genovesi P, Guarino F, Guangzhe L, Hulme PE, Jäger H, Kettle CJ, Krumm F, Langdon B, Lapin K, Lozano V, Le Roux JJ, Novoa A, Nuñez MA, Porté AJ, Silva JS, Schaffner U, Sitzia T, Tanner R, Tshidada N, Vítková M, Westergren M, Wilson JRU, Richardson DM (2020) Global guidelines for the sustainable use of non-native trees to prevent tree invasions and mitigate their negative impacts. NeoBiota 61:65-116. https://doi.org/10.3897/neobiota. 61.58380

Callaham MA, Stanturf JA, Hammond WJ, Rockwood DL, Wenk ES, O’Brien JJ (2013) Survey to evaluate escape of *Eucalyptus* spp. seedlings from plantations in Southeastern USA. Int J For Res 2013:1-10. https://doi.org/10.1155/2013/946374

Calviño-Cancela M, Rubido-Bará M (2013) Invasive potential of *Eucalyptus globulus:* Seed dispersal, seedling recruitment and survival in habitats surrounding plantations. For Eco Man 305:129-137. https://doi.org/10.1016/j.foreco.2013.05.037

Calviño-Cancela M, Lorenzo P, González L (2018) Fire increases *Eucalyptus globulus* seedling recruitment in forested habitats: Effects of litter, shade and burnt soil on seedling emergence and survival. For Eco Man 409:826-834. https://doi.org/10.1016/j.foreco. 2017.12.018

Cleveland WS, Devlin SJ (1988) Locally weighted regression: An approach to regression analysis by local fitting. J Am Stat Assoc 83(403):596-610. https://doi.org/10.1080/01621459.1988. 10478639

Cremer KW (1977) Distance of seed dispersal in eucalypts estimated from seed weights. Aust For Res 7:225-228

Deus E, Silva JS, Larcombe MJ, Catry FX, Queirós L, dos Santos P, Matias H, Águas A, Rego FC (2019) Investigating the invasiveness of *Eucalyptus globulus* in Portugal: Site-scale drivers, reproductive capacity and dispersal potential. Biol Invasions 21:2027-2044. https://doi.org/ 10.1007/s10530-019-01954-6

Dodet M, Collet C (2012) When should exotic forest plantation tree species be considered as an invasive threat and how should we treat them? Biol Invasions 14:1765-1778. https://doi.org/ 10.1007/s10530-012-0202-4

dos Santos P, Matias H, Deus E, Águas A, Silva JS (2015) Fire effects on capsules and encapsulated seeds from *Eucalyptus globulus* in Portugal. Plant Ecol 216:1611-1621. https://doi.org/10.1007/s11258-015-0544-y

Fernandes P, Antunes C, Pinho P, Máguas C, Correia O (2016a) Natural regeneration of *Pinus pinaster* and *Eucalyptus globulus* from plantation into adjacent natural habitats. For Eco Man 378:91-102. https://doi.org/10.1016/j.foreco.2016.07.027

Fernandes PM, Barros AMG, Pinto A, Santos JA (2016b) Characteristics and controls of extremely large wildfires in the western Mediterranean Basin. J Geophys Res Biogeosci 121:2141-2157. https://doi.org/10.1002/2016JG003389

Fernandes P, Máguas C, Correia O (2017) Combined effects of climate, habitat, and disturbance on seedling establishment of *Pinus pinaster* and *Eucalyptus globulus*. Plant Ecol 218(5):501-515. https://doi.org/10.1007/s11258-017-0706-1

Fernandes P, Máguas C, Correia O, González-Moreno P (2018) What drives *Eucalyptus globulus* natural establishment outside plantations? The relative importance of climate, plantation and site characteristics. Biol Invasions 20:1129-1146. https://doi.org/10.1007/s10530-017-1614-y

Garau AM, Ghersa CM, Lemcoff JH, Barañao JJ (2009) Weeds in *Eucalyptus globulus* subsp. *maidenii* (F. Muell) establishment: Effects of competition on sapling growth and survivorship. New For 37:251-264. https://doi.org/10.1007/s11056-008-9121-8

Gassó N, Basnou C, Vilà M (2010) Predicting plant invaders in the Mediterranean through a weed risk assessment system. Biol Invasions 12:463-476. https://doi.org/10.1007/s10530-009-9451-2

Gill AM (1997) Eucalypts and fire: interdependent or independent? In: Williams JE, Woinarski JCZ (eds) Eucalypt ecology. Cambridge University Press: Cambridge, pp 151-1167

Gordon DR, Flory SL, Cooper AL, Morris SK (2012) Assessing the invasion risk of *Eucalyptus* in the United States using the Australian weed risk assessment. Int J For Res 203768. https://doi.org/10.1155/2012/203768

Hartley MJ (2002) Rationale and methods for conserving biodiversity in plantation forests. For Eco Man 155:81-95. https://doi.org/10.1016/S0378- 1127(01)00549-7

ICNF (2017) 10º Relatório provisório de incêndios florestais. Departamento de Gestão de Áreas Públicas e de Protecção Florestal do Instituto da Conservação da Natureza e das Florestas. RIF102017

ICNF (2019) IFN6 - Anexo Técnico. [pdf], 31 pp, versão 1.0 Instituto da Conservação da Natureza e das Florestas, Lisboa

IPMA (2021) - Instituto Português do Mar e da Atmosfera, https://www.ipma.pt/pt/educativa/ tempo.clima/ - Consulted in 2021, February

Jordan G, Potts BM, Wiltshire R (1999) Strong, independent quantitative genetic control of vegetative phase change and first flowering in *Eucalyptus globulus* ssp. *globulus*. Heredity (Edinb). 83:179-187. https://doi.org/10.1046/j.1365-2540.1999.00570.x

Kirkpatrick JB (1975) Natural distribution of *Eucalyptus globulus* Labill. Aust Geogr 13:22-35. https://doi.org/10.1080/00049187508702675

Kirkpatrick JB (1977) Eucalypt invasion in southern California. Aust Geogr 13:387-393. https://doi.org/10.1080/00049187708702717

Lamont BB, LeMaitre DC, Cowling RM, Enright NJ (1991) Canopy seed storage in woody plants. Bot Rev 57:277-317. https://doi.org/10.1007/BF02858770

Larcombe MJ, Silva JS, Vaillancourt RE, Potts BM (2013) Assessing the invasive potential of *Eucalyptus globulus* in Australia: Quantification of wildling establishment from plantations. Biol Invasions 15:2763-2781. https://doi.org/10.1007/s10530-013-0492-1

Marchante H, Morais M, Freitas H, Marchante E (2014) Guia prático para a identificação de plantas invasoras em Portugal. Imprensa da Universidade de Coimbra, Coimbra

Mount AB (1964) The interdependence of the eucalypts and forest fires in Southern Australia. Aust For 28:166-172

Nereu M, Silva JS, Deus E, Nunes M, Potts B (2019) The effect of management operations on the demography of *Eucalyptus globulus* seedlings. For Eco Man 453:117630. https://doi.org/10.1016/j.foreco.2019.117630

Potts BM (1986) Population dynamics and regeneration of a hybrid zone between *Eucalyptus risdonii* Hook-F and *E. amygdalina* Labill. Aust J Bot 34:305-329. https://doi.org/ 10.1071/BT9860305

Pryor LD (1976) The Biology of eucalypts. Edward Arnold (Publishers) Limited, London, UK

Rejmánek M, Richardson DM (2011) Eucalypts. In: Simberloff D, Rejmánek M (eds) Encyclopedia of biological invasions. University of California Press, Berkeley, pp 203-209

Rejmánek M, Richardson DM (2013) Trees and shrubs as invasive alien species - 2013 update of the global database. Divers Distrib 19:1093-1094. https://doi.org/10.1111/ddi.12075

Richardson DM (1998) Forestry trees as invasive aliens. Conserv Biol 12:18-26

Richardson DM, Rejmánek M (2011) Trees and shrubs as invasive alien species - A global review. Divers Distrib 17:788-809. https://10.1111/j.1472-4642.2011.00782.x

Salis M, Ager AA, Finney MA, Arca B, Spano D (2014) Analyzing spatiotemporal changes in wildfire regime and exposure across a Mediterranean fire-prone area. Nat Hazards 71(3):1389-1418. https://doi.org/10.1007/s11069-013-0951-0

Sanz-Elorza M, Dana ED, Sobrino E (2001) Checklist of invasive alien plants in Spain (Iberian Peninsula and Balearic Islands). Lazaroa 22:121-131

Silva JS, Marchante H (2012) Post-fire management of exotic forests. In: Moreira F, Heras J, Corona P, Arianoutsou M (eds) Post-fire management and restoration of Southern European forests. Springer, Dordrecht, pp 223-255

Silva JS, Nereu M, Pinho S, Queirós L, Jesús C, Deus E (2021) Post-fire demography, growth and control of *Eucalyptus globulus* wildlings. Forests 12:156. https://10.3390/f12020156

Soares P, Tomé M, Pereira JS (2007) A produtividade do eucaliptal. In: Alves AM, Pereira JS, Silva JMN (eds) O eucaliptal em Portugal - Impactes ambientais e investigação científica. Lisbon, pp 27-60

Turco M, Jerez S, Augusto S, Tarín-Carrasco P, Ratola N, Jiménez-Guerrero P, Trigo RM (2019) Climate drivers of the 2017 devastating fires in Portugal. Sci Rep 9:13886. https://10.1038/s41598-019-50281-2

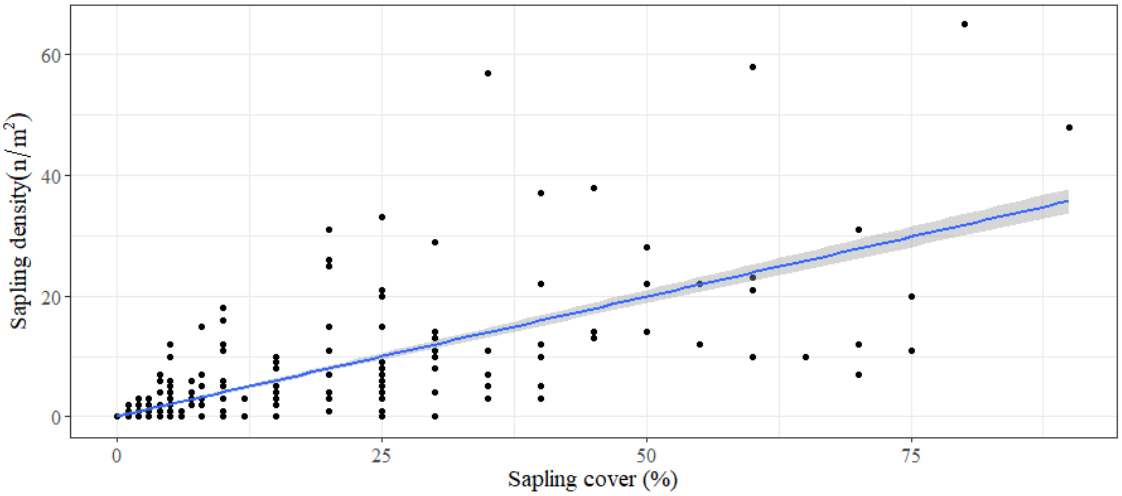
Turnbull JW (1999) Eucalypt plantations. New For 17:37-52. http://doi.org/10.1023/A:1006524911242

Ziller SR, Dechoum MS, Zenni RD (2018) Predicting invasion risk of 16 species of eucalypts using a risk assessment protocol developed for Brazil. Austral Ecol 44(1):28-35. https://doi.org/10.1111/aec.12649

Supporting Information

Supplementary table S2.1 Mean temperature (°C) and Total annual precipitation (mm) from 1931-1960, and mean ± SD altitude (m) in our study sites (Castanheira de Pêra, Castelo de Paiva, Mira, Pampilhosa da Serra, Pedrógão Grande and São Pedro de Alva).

Site	**Mean Temperature (°C)**	**Total annual Precipitation (mm)**	**Mean Altitude (m)**
Castanheira de Pêra (CDP)	10.5	1440	634 ± 73
Castelo de Paiva (CSP)	12.4	1300	326 ± 109
Mira (M)	14.6	952	37 ± 17
Pampilhosa da Serra (PS)	12.7	1300	588 ± 96
Pedrógão Grande (PG)	14.3	1140	415 ± 63
São Pedro de Alva (SPA)	13.2	1078	226 ± 58



Supplementary figure S2.1: Spearman correlation of *E. globulus* sapling cover (%) and sapling density (n/m^2) (r = 0.88).

Chapter 3

CLIMATE AND LOCAL FACTORS INFLUENCE *EUCALYPTUS GLOBULUS* ESTABLISHMENT AFTER OFF-SEASON FIRES

Anjos A, Jesus JG, Marques C, Borralho N, Valente C, Correia O, Máguas C, Chozas S (2022) Climate and local factors influence *Eucalyptus globulus* establishment after off-season fires. New For 54:981-992. https://doi.org/10.1007/s11056-022-09950-w

Abstract

Eucalyptus globulus Labill. is one of the most widely planted hardwood species worldwide. This species occupies a quarter of the country's forested area in Portugal, so there is a growing concern about its post-fire dispersal. Although it is generally recognised that fire promotes *E. globulus* natural regeneration and that precipitation and topography influence recruitment, little is known about the role of post-fire conditions on the establishment of the species following off-season fires. We examine how post-fire conditions affect *E. globulus* natural regeneration and invasive potential. Sapling establishment was assessed in plantations and under old and large isolated *Eucalyptus* trees (seed-trees) following two off-season fire events (2017 June and October fires). Abiotic and biotic local factors affected differently sapling establishment in plantations and under seed-trees. In plantations, sapling cover was more influenced by tree-related traits (age and height), whereas the conditions beneath seed-trees mitigated the impact of harsh conditions on saplings. In both cases, post-fire climatic conditions constrained natural regeneration, with less impact under seed-trees, reinforcing their importance in establishment success. Thus, isolated seed-trees should be considered while managing the species' unplanned spread.

3.1 Introduction

Eucalyptus globulus Labill. (Tasmanian blue gum), endemic to the Australian mainland and Tasmania (Jacobs 1979), is an important economic hardwood species in Portugal (Tomé *et al*. 2021) due to its fast growth, vigour, and suitability as pulpwood for the paper industry (Jacobs 1979). Some authors consider this species invasive outside of its natural range (Sanz-Elorza *et al*. 2001; Gassó *et al*. 2010; Gordon *et al*. 2012; Rejmánek and Richardson 2013; Ziller *et al*. 2018), including in Portugal (Marchante *et al*. 2014). However, several studies have shown that its dispersal potential is low and limited to the first 15 m beyond the plantation boundary (Calviño-Cancela and Rubido-Bará 2013; Larcombe *et al*. 2013; Fernandes *et al*. 2016). Presently, *E. globulus* is not included in Portugal's official list of invasive plants. This species is highly adapted to post-fire regeneration, accounting for the prolific germination of seeds released from canopy seed banks (Lamont *et al*. 1991), which are protected from heat by woody capsules (Gill 1981; dos Santos *et al*. 2015). After a fire event, seeds may find favourable conditions to germinate and establish due to the absence of competition and allelopathic effects, higher light availability, and nutrients in ash beds

(Chambers and Attiwill 1994; Gill 1997). Therefore, fire occurrence can potentially increase the invasive capacity of *E. globulus* (Águas *et al*. 2014; Calviño-Cancela *et al*. 2018; Anjos *et al*. 2021).

Over the last four decades, *E. globulus* has been planted in Portugal, occupying 26% (about 850 000 ha) of the country's forested area (ICNF 2019). Most of the eucalypt area is production plantation, managed in 10–12-year rotation cycles and subjected to weed control (Alves *et al*. 2007). Unfortunately, some plantations are unmanaged, and these could potentially promote the establishment of new populations (Águas *et al*. 2017). In addition, old and large isolated trees (hereafter referred to as seed-trees) are used to delimit property boundaries. These trees have higher capsule production than those in plantations and are important sources of natural establishment (i.e., plants that have naturally spread from the mother tree) (Anjos *et al*. 2021).

Forest fires have become a global problem due to climate and land-use changes. Portugal presents the highest percentage of burnt area in Europe and fire is one of the main environmental concerns in the country (Mateus and Fernandes 2014; Turco *et al*. 2016). Additionally, global warming has profoundly changed Portugal's fire regime, increasing the frequency and intensity of forest fires (Turco *et al*. 2019; IPCC 2021). These conditions have led to severe fires outside the summer months, starting early in spring and extending until late autumn (Castellnou *et al*. 2018; Sánchez-Benítez *et al*. 2018). For example, the combination of heat waves with high temperatures, low humidity, and strong winds (Turco *et al*. 2019), along with the vegetation structure and biomass accumulation (Fernandes 2009), led to the catastrophic fires that occurred in Portugal on June 17 (early in the season) and on October 15 (late in the season) in 2017. In this context, Anjos *et al*. (2021) found that autumn fires (October) in unmanaged stands and the presence of seed-trees could promote *E. globulus* expansion through natural recruitment. Indeed, it is known that *E. globulus* recruitment and invasiveness may be influenced by precipitation and frost (Catry *et al*. 2015), factors that are altered by climate change (Richardson and Rejmánek 2011), as well as by mother tree age (Águas *et al*. 2017), slope steepness and topographic characteristics (Catry *et al*. 2015; Queirós *et al*. 2020). Thus, how *E. globulus* will respond to off-season post-fire conditions is of concern.

We assessed the effect of climate and local conditions after the June and October fires on *E. globulus* natural regeneration by examining sapling establishment in managed and unmanaged plantations and under seed-trees. In addition, we determine if natural regeneration after off-season fires occurs independently of post-fire conditions and if saplings in plantations and under isolated trees respond differently.

3.2 Material and Methods

3.2.1 Study area and data collection

This study was conducted in central and northern Portugal, in sites affected by the 2017 catastrophic wildfires: Castanheira de Pêra (CDP) and Pedrógão Grande (PG), where the fire occurred in June (total burnt area of ca. 52 000 ha), and Castelo de Paiva (CSP), Mira (M), Pampilhosa da Serra (PS) and São Pedro de Alva (SPA) that burnt in October (total burnt area of 190 000 ha, ICNF 2019) (Fig. 3.1a). According to the Köppen-Geiger classification, all studied areas are classified as a warm-summer Mediterranean climate (Csb), representing a warm temperate climate with dry and warm summers (Kottek *et al*. 2006; IPMA 2021). The altitude varies from 6 to 699 m. An aridity index was estimated considering the climatic conditions for the three months after each fire event (adapted from De Martonne 1926). Post-fire climatic conditions were contrasting: while after the June fire, the mean total precipitation was 35.7 mm, and mean minimum and maximum temperatures were 14.7 °C and 28.7 °C, after the October fire, precipitation was 287.7 mm, and mean minimum and maximum temperatures were 4.8 °C, and 13.5 °C, respectively (CRU-TS 4.03; Harris *et al*. 2014) downscaled with WorldClim 2.1 (Fick and Hijmans 2017) (Fig. 3.1b, c and Supplementary Table S3.1).

Sampling was performed from October to December 2018, about one year after the fires. At each site, *E. globulus* plantations were selected according to the following criteria: (1) they were located more than 70 m away from other plantations and no *E. globulus* individuals or seed-trees occurred within the matrix between plantations; and (2) trees had capsules in their canopy representing current reproductive capacity. All studied plantations had at least 0.5 ha with easy access, and their canopies were entirely burnt. Isolated large seed-trees were selected when: (1) they were at least 70 m away from other seed-trees; (2) they were at least 100 cm CBH (Circumference at Breast Height); and (3) they presented a burnt canopy.

Three managed and three unmanaged *E. globulus* plantations along with 14 seed-trees were sampled within the area affected by the June fire. Seven managed and five unmanaged plantations and 28 isolated seed-trees were selected in the October fire-affected-areas. At least three transects were sampled at plantation edges at each site and located 15 m apart (plantation transects) (Fig. 3.1e). A minimum of two and a maximum of four transects were sampled around seed-trees (seed-tree transects) (Fig. 3.1d). The total number of transects sampled is summarised in Table 3.1.

Table 3.1 Total number of transects sampled within the study area, including plantation and seed-tree transects, both managed and unmanaged, affected by the 2017 June and October fire events.

	June fire		**October fire**	
	Managed	**Unmanaged**	**Managed**	**Unmanaged**
Plantation	10	13	30	21
Seed-tree	27	20	39	45

Transects perpendicular to the plantation edge and seed-trees were sampled using 3 × 3 m plots (Fig. 3.1d and e). Regarding plantations, transects were performed outside and inside plantation boundaries. Outside the plantation and for seed-tree transects, the first plot was established at 0 or 3 m, and then plots were sampled at 3 m intervals (adapted from Callaham *et al*. 2013). Transect sampling finished when two consecutive plots had no *E. globulus* saplings, marking the local limit of seed dispersal. Then, within the plantation, three alternate plots were sampled (starting at the symmetrical distance of the outside transect, i.e., if the first outside plot was sampled at 3 m, the same occurred in the inside plot) (Fig. 3.1e).

Each transect was classified as either a plantation or a seed-tree transect, by the date the fire occurred (June or October) and by management type (managed or unmanaged, see Table 3.2). Regarding seed-trees, management type was classified at the transect level since these trees are normally used to limit properties or located near roads, making surrounding areas subjected to different management regimes. The dominant tree species in the surrounding matrix was used to classify the matrix type. Sampled plots located inside plantations were classified as *E. globulus* habitats.

In each plot, the percentage of cover of *E. globulus* saplings, vegetation (herbs and shrubs), trees, litter, stone, and bare soil were visually assessed, adapted from Larcombe *et al*. (2013). The CBH of *E. globulus* trees in plantations and seed-trees was measured as a proxy of tree age. *Eucalyptus globulus* saplings from seed germination were distinguished from planted individuals based on their position and size relative to the planted trees (unkempt plantation lines) that were mostly already resprouting. Each plot was classified regarding its slope relative to the plantation edge or seed-tree as flat (no inclination), moderate, moderate down (slope downward) and moderate up (slope upward). All variables are summarised in Table 3.2.

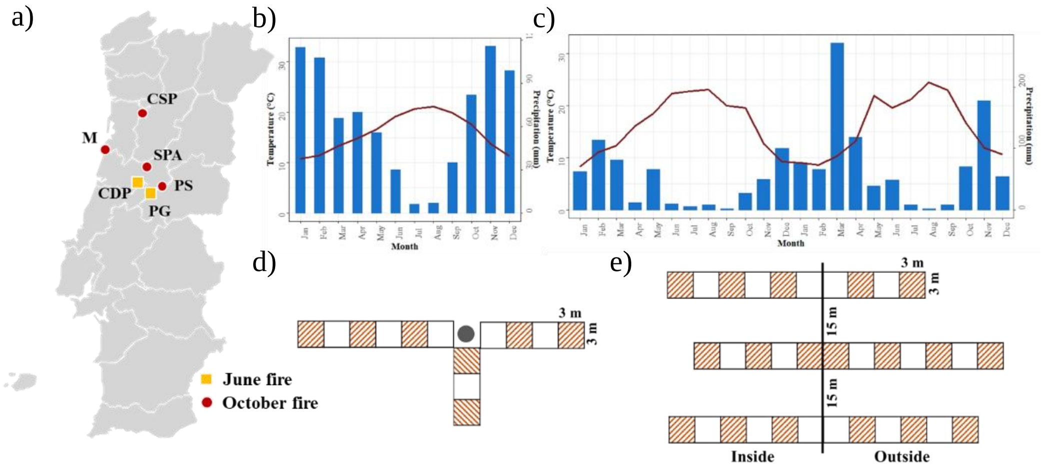


Figure 3.1 Study area location, climate data and sampling procedure. a Sites sampled in Castanheira de Pêra (CDP) and Pedrógão Grande (PG), affected by the 2017 June fire, represented with a yellow square, and Castelo de Paiva (CSP), Mira (M), São Pedro de Alva (SPA) and Pampilhosa da Serra (PS), affected by 2017 October fires, represented with a red circle; monthly average temperature [°C] (line) and monthly average precipitation [mm] (bars) b from 1982 to 2012 in Portugal (Climate-data.org) and c from January 2017 to December 2018 in Portugal, the period including fire dates and data collection; schematic diagrams of d seed-tree transects and e plantation transects, established with 3 × 3 m plots; only coloured plots were sampled.

Table 3.2 Variables used as predictors for the natural regeneration of *E. globulus* saplings after the 2017 June and October fires.

Variable	Data description	Data type	Assessed for
Adjacent habitat	*Eucalyptus globulus* (applied in plots located inside plantations), Mixed *E. globulus*, *Pinus pinaster*, Mixed *P. pinaster*, Mixed *Acacia* sp., Mixed *Quercus* sp. or Shrubland	Categorical	Transect
Altitude	From 6 to 699 meters	Continuous	Plot
Bare soil	% cover	Continuous	Plot
CBH	Circumference at breast height of *E. globulus* trees	Continuous	Plantation or Seed-tree
De Martonne aridity index adapted	Aridity for the three months after the 2017 June and October fire events; from 0.05 to 0.87 $1/\ (\frac{Total\ precipitation}{Mean\ temperature+10})$	Continuous	Plantation or Seed-tree
Distance	Distance to plantation edge or seed-tree	Continuous	Plot
Litter	% cover	Continuous	Plot
Management	Managed - ≤ 3 trunks per tree, and if large trunks of burnt shrubs or trees were not observed Unmanaged – > 3 trunks per tree, and if large trunks of burnt shrubs or trees were observed	Categorical	Plantation; Transect (in Seed-trees)
Slope	Flat, Moderate, Moderate down or Moderate up: Down - when the plantation or seed-tree transects were sampled downwards Up - the plantation or seed-tree transects were sampled upwards	Categorical	Plot
Stone	% cover	Continuous	Plot
Trees	% cover	Continuous	Plot
Vegetation	% cover of herbaceous and shrubs	Continuous	Plot

2.2 Data analysis

To assess the factors influencing *E. globulus* regeneration after the off-season fires, sapling cover was used as a response variable in a Generalized Linear Model (GLM) with a negative binomial distribution. The explanatory variables were adjacent habitat, altitude, bare soil cover, De Martonne aridity index adapted, distance to plantation or seed-tree, litter cover, management type, mean CBH, slope, stone cover, tree cover and vegetation cover. Sapling establishment in plantations and under seed-trees was assessed separately since differences between these categories were already evaluated in previous work (Anjos *et al*. 2021). Zero inflation was used to account for the overdispersion of the data associated with a large number of zeros in the response variable.

A multi-model inference approach was used to identify the drivers of regeneration using the MuMIn package. Through the "dredge function", model selection was performed across all possible model subsets using Akaike’s Information Criterion (AICc) based on maximum likelihood. Post-hoc standardization (method pseudo) of the parameters was performed to compare variables within each model (Siegel 2016).

Variance Inflation Factors (VIF) were used to test for model multicollinearity (Zuur *et al*. 2009). The selected variables included in the models were not correlated (Spearman correlation coefficient < 0.70 and VIF < 0.5).

Data analysis was done using packages MASS (Venables and Ripley 2002) and MuMIn (Bartoń 2022) in R statistical software version 0.3.6.1 (R Core Team 2020).

3.3 Results

Saplings in plantations and under seed-trees responded differently to post-fire and local conditions. In plantation transects, altitude, aridity, distance to the plantation edge, and stone cover were significantly associated with lower *E. globulus* sapling cover. In contrast, mixed *P. pinaster* matrix*, E. globulus* tree CBH and moderate slope were associated with a higher *E. globulus* sapling cover in plantations (Table 3.3).

Sapling cover in plantations was lowest and most affected by distance to plantation edge (R = - 0.97, Fig. 3.2), followed by altitude (R = - 0.83) and aridity (R = - 0.67). In contrast, the mean *E. globulus* tree CBH was the most associated with sapling establishment (R = 0.92), unmanaged conditions and moderate slope (R = 0.72 and 0.60, respectively).

Table 3.3 Summary of the best model assessing the influence of variables on sapling cover from *E. globulus* plantations after the 2017 June and October fires, using Akaike's Information Criterion (AICc) based on maximum likelihood. Generalized Linear Model (GLM) coefficients, standard errors, the z statistic and the associated probabilities are presented (ns = non-significant; * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001).

	Estimate	**Std. Error**	**z value**	**Pr(> |z|)**
(Intercept)	-0.604	0.348	-1.73	ns
Altitude	-0.004	0.001	-7.53	***
Aridity index	-1.982	0.328	-6.05	***
Distance to plantation edge	-0.062	0.005	-11.65	***
E. globulus CBH	0.048	0.007	7.02	***
Litter cover	-0.007	0.002	-2.80	**
Management [Unmanaged]	1.434	0.259	5.52	***
Slope [Moderate]	1.431	0.280	5.13	***
Slope [Moderate down]	0.060	0.202	0.30	ns
Slope [Moderate up]	-0.133	0.380	-0.35	ns

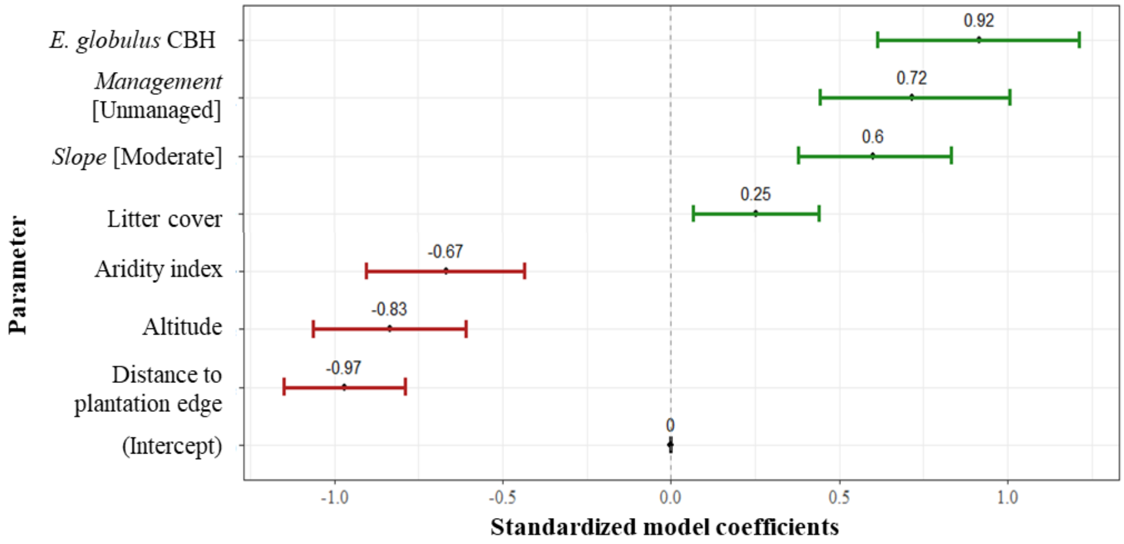


Figure 3.2 Standardized model-averaged coefficients (means and 95% confidence intervals) of variables influencing sapling cover assessed in *E. globulus* plantations after the 2017 June and October fires. Negative values represent detrimental factors (in red) and positive values represent beneficial factors (in green).

Under isolated seed-trees, all variables in the model were associated with lower sapling cover except unmanaged conditions (R = 0.63): the stone cover was associated with the lowest sapling cover (R = - 0.67), followed by vegetation cover (R = - 0.56), altitude (R = -

0.41), aridity index (R = - 0.28) and moderate down-slopes (R = - 0.27) (Table 3.4 and Fig. 3.3).

Table 3.4 Summary of the best model assessing the influence of variables on sapling cover assessed under *E. globulus* seed-trees after the 2017 June and October fires, using Akaike's Information Criterion (AICc) based on maximum likelihood. Generalized Linear Model (GLM) coefficients, standard errors, the z statistic and the associated probabilities are presented (ns = non-significant; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$).

	Estimate	**Std. Error**	**z value**	**Pr(> |z|)**
(Intercept)	3.087	0.191	16.18	***
Altitude	-0.002	0.001	-3.53	***
Aridity index	-0.780	0.280	-2.78	**
Management [Unmanaged]	1.274	0.166	7.66	***
Slope [Moderate]	0.271	0.197	1.37	**Ns**
Slope [Moderate down]	-1.137	0.373	-3.05	**
Slope [Moderate up]	0.635	0.330	1.93	**Ns**
Stone cover	-0.019	0.003	-6.53	***
Vegetation cover	-0.016	0.002	-6.55	***

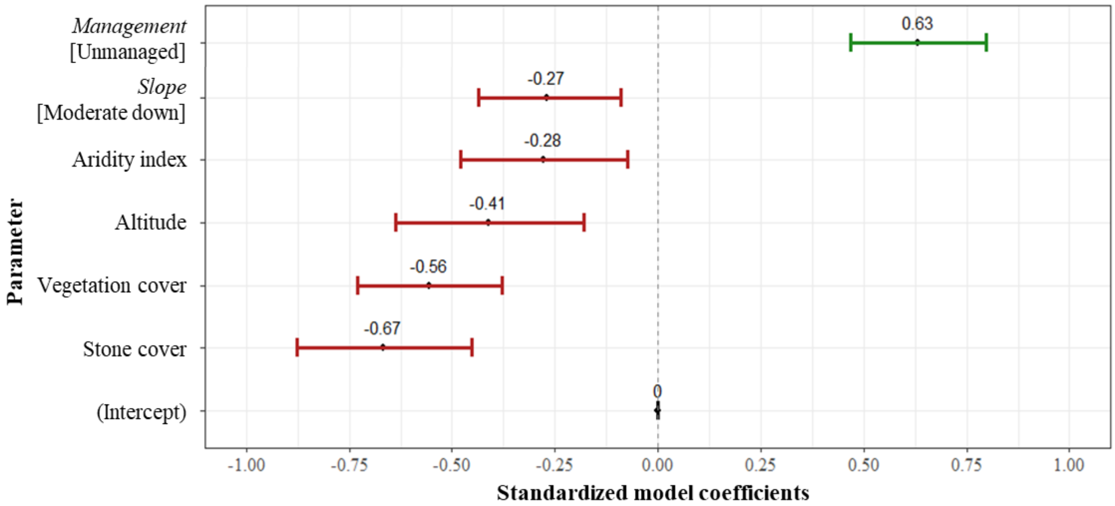


Figure 3.3 Standardized model-averaged coefficients (means and 95% confidence intervals) of variables influencing sapling cover assessed under *E. globulus* seed-trees after the 2017 June and October fires. Negative values represent detrimental factors (in red) and positive values represent beneficial factors (in green).

3.4 Discussion

Eucalyptus globulus saplings on plantations and from seed-trees are influenced by different variables and respond differently to post-fire climatic and local conditions.

Our findings revealed that, in plantations, tree-related traits affected natural regeneration: trees with higher CBH (i.e., older trees found in unmanaged plantations) presented higher sapling cover mainly due to their greater reproductive output (Larcombe *et al*. 2013; Águas *et al*. 2017). *Eucalyptus globulus* is a serotinous species that accumulates mature capsules in the canopy for several months or even years (Lamont *et al*. 1991); in this sense, the seed dispersal capacity increases with age (Águas *et al*. 2017; Anjos *et al*. 2021). However, post-fire dispersal is severely limited by increasing distance to the plantation edge, mainly due to the lack of seed dispersal mechanisms, making *E. globulus* dispersal capacity highly dependent on parent-tree height (Cremer 1977). Interestingly, the effect of tree CBH on sapling cover was not observed in seed-trees (far older than unmanaged plantation trees), indicating the importance of these old and isolated trees as a seed source for *E. globulus* dispersal, regardless of their age.

In addition, post-fire climatic conditions were also relevant for sapling cover for both plantations and seed-trees. Precipitation and temperature registered three months after the June and October fires, corresponding to summer and autumn, respectively, influenced this species' establishment with greater aridity resulting in lower sapling cover. *Eucalyptus globulus* germination and establishment are highly dependent on soil moisture (Larcombe *et al*. 2013; Fernandes *et al*. 2017; Queirós *et al*. 2020), therefore favourable conditions are more likely to occur after autumn (October) fires, corresponding to the wetter season in Portugal. This corroborates the observation of Silva *et al*. (2021), who found the greatest wildling density for *E. globulus* in Portugal after the 2017 October fire. Again, saplings resulting from seed-trees were less influenced by climatic conditions (aridity influence was halved), suggesting that these trees might ameliorate microclimatic conditions by reducing soil temperatures and providing mild shade under their canopy, facilitating establishment. These microclimatic conditions, however, are expected to enhance vegetation development, which competes with *E. globulus* saplings and lowers their establishment. Stone cover hampered *E. globulus* establishment the most as stones are a natural physical barrier to seed germination. This is significant given the lack of endosperm reserves in *E. globulus* seeds (Calviño-Cancela *et al*. 2018). Furthermore, in contrast to the effect of slope on saplings from seed-trees, slope shallowness had a substantially beneficial effect on saplings from plantations. While plantation edges may improve nutrient retention and water retention on moderate slopes, single seed-trees do not prevent nutrient lixiviation and reduce seed accumulation. As a result, regardless of seed germination capacity, sapling establishment may be compromised under these sloping and unfavourable conditions.

In summary, as previously shown by Anjos *et al*. (2021), there is a higher natural regeneration in seed-tree adjacent areas. However, despite unmanaged conditions that increased sapling establishment in this study, all the modelled variables reduced sapling cover. Thus, the presence of seed-trees, unaffected by the adverse effects of local factors, is responsible for higher sapling cover and ultimately promotes the establishment of wildlings of this species.

Conclusions

Local factors following off-season fires provide severe ecological restrictions to *E. globulus* natural establishment. Sapling establishment in plantations and under seed-trees was determined by different factors: whereas tree-related characteristics (tree CBH and distance, linked to height) affected sapling cover in plantations, saplings under seed-trees were able to overcome harsh conditions, including dryness. However, because these factors are highly variable and site-dependent, *E. globulus* behaviour after fire remains challenging to predict and characterise, especially under a climate change scenario associated with an increasingly unpredictable fire season and regimes. Nevertheless, because of their importance as a propagule source, this study reinforces that seed-trees must be considered in management strategies.

References

Águas A, Ferreira A, Maia P, Fernandes PM, Roxo L, Keizer J, Silva JS, Rego FC, Moreira F (2014) Natural establishment of *Eucalyptus globulus* Labill. in burnt stands in Portugal. For Eco Man 323:47-56. https://doi.org/10.1016/j.foreco. 2014.03.012

Águas A, Incerti G, Saracino A, Lanzotti V, Silva JS, Rego FC, Mazzoleni S, and Bonanomi G (2017) Fire effects on litter chemistry and early development of *Eucalyptus globulus*. Plant Soil 422: 495-514. https://doi.org/10.1007/s11104-017-3419-2

Alves AM, Pereira JS, Silva JN (2007) O eucaliptal em Portugal – Impactes ambientais e investigação científica. ISAPress, Lisbon

Amatulli G, Camia A, San-Miguel-Ayanz J (2013) Estimating future burned areas under changing climate in the EU-Mediterranean countries. Sci Total Environ 450-451:209-222. https://doi.org/10.1016/j.scitotenv.2013.02.014

Anjos A, Fernandes P, Marques C, Borralho N, Valente C, Correia O, Máguas C and Chozas S (2021) Management and fire, a critical combination for *Eucalyptus globulus* dispersal. For Eco Man 490:119086. https://doi.org/10.1016/j.foreco. 2021.119086

Bartoń K (2022) MuMIn: Multi-Model Inference. R package version 1.46.0. https://CRAN.R-project.org/package=MuMIn

Brundu G, Pauchard A, Pyšek P, Pergl J, Bindewald AM, Brunori A, Canavan S, Campagnaro T, Celesti-Grapow L, de Dechoum MS, Dufour-Dror JM, Essl F, Flory SL, Genovesi P, Guarino F, Guangzhe L, Hulme PE, Jäger H, Kettle CJ, Krumm F, Langdon B, Lapin K, Lozano V, Le Roux JJ, Novoa A, Nũnez MA, Porté AJ, Silva JS, Schaffner U, Sitzia T, Tanner R, Tshidada N, Vítková M, Westergren M, Wilson JRU, Richardson DM (2020) Global guidelines for the sustainable use of non-native trees to prevent tree invasions and mitigate their negative impacts. NeoBiota 61:65-116. https://doi.org/10.3897/neobiota.61.58380

Callaham MA, Stanturf JA, Hammond WJ, Rockwood DL, Wenk ES, O’Brien JJ (2013) Survey to evaluate escape of *Eucalyptus* spp. seedlings from plantations in Southeastern USA. Int J For Res:1-10. https://doi.org/10.1155/2013/946374

Calviño-Cancela M, Rubido-Bará M (2013) Invasive potential of *Eucalyptus globulus*: Seed dispersal, seedling recruitment and survival in habitats surrounding plantations. For Eco Man 305:129-137. https://doi.org/10.1016/j.foreco.2013.05.037

Calviño-Cancela M, Lorenzo P, González L (2018) Fire increases *Eucalyptus globulus* seedling recruitment in forested habitats: Effects of litter, shade and burnt soil on seedling emergence and survival. For Eco Man 409:826-834. https://doi.org/10.1016/j.foreco.2017.12.018

Castellnou M, Guiomar N, Rego F, Fernandes P (2018) Fire growth patterns in the 2018 mega fire episode of October 15, central Portugal. Advances in Forest Fire Research:447-453. https://doi.org/10.14195/978-989-26-16-506_48

Castro-Díez P, Alonso Á, Saldaña-López A, Granda E (2021) Effects of widespread non-native trees on regulating ecosystem services. Sci Total Environ 778:146141. https://doi.org/10.1016/j. scitotenv.2021.146141

Catry FX, Moreira F, Deus E, Silva JS, Águas A (2015) Assessing the extent and the environmental drivers of *Eucalyptus globulus* wildling establishment in Portugal: results from a countrywide survey. Biol Invasions 17: 3163-3181. https://doi.org/10.1007/s10530-015-0943-y

Chambers DP, Attiwill PM (1994) The ash-bed effect in *Eucalyptus regnans* forest – chemical, physical and microbiological changes in soil after heating or partial sterilization. Aust J Bot 42:739-749. https://doi.org/10.1071/BT9940739

Cremer KW (1977) Distance of seed dispersal in eucalypts estimated from seed weights. Aust For Res 7:225-228. https://doi.org/10.1071/BT9940739

De Martonne E (1926) Aerisme, et índices d'aridite. Comptesrendus de L'Academie des Sciences 182:1395-1398

dos Santos P, Matias H, Deus E, Águas A, Silva JS (2015) Fire effects on capsules and encapsulated seeds from *Eucalyptus globulus* in Portugal. Plant Ecol 216:1611-1621. https://doi.org/10.1007/s11258-015-0544-y

FAO (2010) Global forest resource assessment. Forestry Department. Food and Agriculture Organization of the United Nations, Rome

Fernandes PM (2009) Combining forest structure data and fuel modelling to assess fire hazard in Portugal. Ann For Sci 66:415. https://doi.org/10.1051/forest/2009013

Fernandes P, Antunes C, Pinho P, Máguas C, Correia O (2016) Natural regeneration of *Pinus pinaster* and *Eucalyptus globulus* from plantation into adjacent natural habitats. For Eco Man 378:91-102. http://doi.org/ 10.1016/j.foreco.2016.07.027

Fernandes P, Máguas C, Correia O (2017) Combined effects of climate, habitat, and disturbance on seedling establishment of *Pinus pinaster* and *Eucalyptus globulus*. Plant Ecol 218:501-515. https://doi.org/10.1007/s11258-017-0706-1

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. Int J Clim 37:4302-4315. https://doi.org/10.1002/joc.5086

Florence RG (1996) Ecology and silviculture of eucalypt forests. CSIRO, Collingwood

Gassó N, Basnou C, Vilà M (2010) Predicting plant invaders in the Mediterranean through a weed risk assessment system. Biol Invasions 12:463-476. https://doi.org/10. 1007/s10530-009-9451-2

Gill AM (1997) Eucalypts and fire: interdependent or independent? In: Williams JE, Woinarski JCZ (eds) Eucalypt ecology. Cambridge University Press: Cambridge, UK, pp 51-1167

Gill AM (1981) Adapted response of Australian vascular plant species to fires. In: Gill AM, Groves RH, Noble IR (eds) Fire and the Australian biota. Australian Academy of Science, Canberra, pp. 243-271

Golivets M, Wallin KF (2018) Neighbour tolerance, not suppression, provides competitive advantage to non-native plants. Ecol Lett 21:745-459. https://doi-org/10.1111/ele.12934

Gordon DR, Flory SL, Cooper AL, Morris SK (2012) Assessing the invasion risk of *Eucalyptus* in the United States using the Australian weed risk assessment. Int J For Res 2012:203768. https://doi.org/10.1155/2012/203768

Harris I, Jones PD, Osborn TJ, Lister DH (2014) Updated high-resolution grids of monthly climatic observations - The CRU TS3.10 Dataset. Int J Climatol 34:623-642. https://doi.org/10.1002/joc.3711

ICNF (2019) IFN6 – Anexo Técnico. [pdf], 31 pp, versão 1.0 Instituto da Conservação da Natureza e das Florestas, Lisboa

IPCC (2021) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. https://doi.org/10.1017/9781009157896

IPMA (2021) https://www.ipma.pt/pt/educativa/tempo.clima/ (accessed in June 2021)

Jacobs MR (1979) Eucalypts for planting. FAO, Rome

Korboulewsky N, Tétégan M, Besnault A, Cousin I (2010) Do rock fragments participate to plant water and mineral nutrition? Geophys Res Abstr 12

Kottek M, Grieser J, Beck C, Rudolf B, Rubel F (2006). World map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift 15:259-263. https://doi.org/10.1127/0941-2948/2006/0130

Lamont BB, LeMaitre DC, Cowling RM, Enright NJ (1991) Canopy seed storage in woody plants. Bot Rev 57:277-317

Larcombe MJ, Silva JS, Vaillancourt RE, Potts BM (2013) Assessing the invasive potential of *Eucalyptus globulus* in Australia: Quantification of wildling establishment from plantations. Biol. Invasions 15:2763-2781. https://doi.org/10.1007/s10530-013-0492-1

Lionello P, Malanotte-Rizzoli P, Boscolo R, Alpert P, Artale V, Li L, Luterbacher J, May W, Trigo R, Tsimplis M, Ulbrich U, Xoplaki E (2006) The Mediterranean climate: An overview of the main characteristics and issues. Dev Earth Environ Sci 4:1-26. https://doi.org/10.1016/S1571-9197(06)80003-0

Marchante H, Morais M, Freitas H, Marchante E (2014). Guia prático para a identificação de plantas invasoras em Portugal. Imprensa da Universidade de Coimbra, Coimbra

Mateus P, Fernandes P (2014) Chapter 4 - Forest fires in Portugal: Dynamics, causes and policies. In: Reboredo F (ed) Forest context and policies in Portugal, present and future challenges. World Forests series, Springer, pp 97-114

Pausas JG, Vallejo VR (1999) The role of fire in European Mediterranean Ecosystems. In: Chuvieco E. (ed.): Remote sensing of large wildfires in the European Mediterranean basin, pp 3-16

Queirós L, Deus E, Silva JS, Vicente J, Ortiz L, Fernandes PM, Castro-Díez P (2020) Assessing the drivers and recruitment potential of *Eucalyptus globulus* in the Iberian Peninsula. For Eco Man 466:118147. https//doi.org/10.1016/j.foreco.2020.118147

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rejmánek M, Richardson DM (2013) Trees and shrubs as invasive alien species - 2013 update of the global database. Divers Distrib 19:1093-1094. https://doi.org/10.1111/ddi.12075

Richardson DM, Rejmánek M (2011) Trees and shrubs as invasive alien species – A global review. Divers Distrib 17:788-809. https://doi.org/10.1111/j.1472-4642.2011.00782.x

Sánchez-Benítez A, García-Herrera R, Barriopedro D, Sousa PM, Trigo RM (2018) June 2017: The Earliest European Summer Mega-heatwave of Reanalysis Period. Geophys Res Lett 45:1955-1962. https://doi.org/10.1002/2018GL077253

Sanz-Elorza M, Dana ED, Sobrino E (2001) Checklist of invasive alien plants in Spain (Iberian Peninsula and Balearic Islands). Lazaroa 22:121-131

Shakesby RA (2011) Post-wildfire soil erosion in the Mediterranean: Review and future research directions. Earth-Sci Rev 105:71-100. https://doi.org/10.1016/j.earscirev.2011.01.001

Siegel AF (2016) Chapter 12 - Multiple regression: Predicting one variable from several others in practical business statistics (Seventh Edition). Academic Press, pp 355-418

Silva JS, Nereu M, Pinho S, Queirós L, Jésus C, Deus E (2021) Post-fire demography, growth, and control of *Eucalyptus globulus* wildlings. Forests 12:156. https://doi.org/10.3390/f12020156

Tomé M, Almeida MH, Barreiro S, Branco MR, Deus E, Pinto G, Silva JS, Soares P, Rodríguez-Soalleiro R (2021) Opportunities and challenges of *Eucalyptus* plantations in Europe: The Iberian Peninsula experience. Eur J For Res 140:489-510. https:// doi.org/10.1007/s10342-021-01358-z

Turco M, Bedia J, Di Liberto F, Fiorucci P, von Hardenberg J, Koutsias N, Llasat MC, Xystrakis F, Provenzale A (2016) Decreasing fires in Mediterranean Europe. PLoS ONE 11:e0150663

Turco M, Jerez S, Augusto S, Tarín-Carrasco P, Ratola N, Jiménez-Guerrero P, Trigo RM (2019) Climate drivers of the 2017 devastating fires in Portugal. Sci Rep 9:13886. https://doi.org/10.1038/s41598-019-50281-2

Venables WN, Ripley BD (2002) Modern applied statistics with S. Fourth edition. Springer New York

Ziller SR, Dechoum MS, Zenni RD (2018) Predicting invasion risk of 16 species of eucalypts using a risk assessment protocol developed for Brazil. Austral Ecol 44(1):28-35. https://doi.org/10.1111/aec.12649

Zuur A, Ieno E, Walker N, Saveliev AA, Smith G (2009) Mixed effects models and extensions in ecology with R. Springer New York

Supporting Information

Supplementary table S3.1: Climatic conditions registered in the three months after each fire (Total mean precipitation (mm), Mean minimum temperature (ºC), Mean maximum temperature (ºC), Mean temperature (ºC), mean De Martonne aridity index adapted) and mean Altitude (m) in Castanheira de Pêra (CDP) and Pedrógão Grande (PG), affected by 2017 June fire, and Castelo de Paiva (CSP), Mira (M), São Pedro de Alva (SPA) and Pampilhosa da Serra (PS), affected by 2017 October fires. Data obtained in CRU-TS 4.03; Harris *et al.* 2014) downscaled with WorldClim 2.1 (Fick and Hijmans 2017).

			Temperature (ºC)				
Site	**Fire date**	**Total Precipitation (mm)**	**Mean minimum**	**Mean maximum**	**Mean**	**De Martonne aridity index adapted**	**Altitude (m)**
PG	June	35.7	14.7	28.7	21.7	0.87	408
CDP	June	42.2	14.4	27.8	21.1	0.72	568
CSP	October	387.7	5.3	14.4	9.9	0.05	288
M	October	270.2	6.4	16.2	11.3	0.08	36
SPA	October	348.5	5.7	15.6	10.7	0.06	213
PS	October	348.3	4.8	13.5	9.2	0.06	650

Chapter 4

EXPLORING REPRODUCTIVE BIOLOGY IN *EUCALYPTUS GLOBULUS* CLONES UNDER DISTINCT CLIMATIC CONDITIONS

Anjos A, Jesus JG, Marques C, Borralho N, Valente C, Araújo JA, Máguas C, Correia O, Chozas S (2023) Exploring reproductive biology in *Eucalyptus globulus* clones under distinct climatic conditions. Discover Agriculture 1(6). https://doi.org/10.1007/s44279-023-00006-x

Abstract

Despite the great economic benefits, one of the major threats of introducing species into exotic ranges is their ability to regenerate and spread to novel environments. For example, *Eucalyptus globulus* plantations have been established in many parts of the world, using seedlings or clonal material, the latter often from advanced generation tree improvement programs. However, major concerns exist about this species' ability to invade non-native areas. Therefore, it is necessary to study whether breeding programs affect this species' reproductive biology to assess the risk of invasiveness of these improved trees. In this sense, this work compared the *E. globulus* phenological behaviour and reproductive structures production of three clonal and one seedling-based stand across two distinct climatic zones (mesic and xeric) in Portugal. Through binocular observation, four different stands (seedlings descending from open-pollinated crosses and three clonal-stands) were monitored every three weeks for different reproductive phenological stages, namely the presence of flowers and capsules. Clonal stands presented a shorter flowering season, less abundant reproductive structures, and higher phenological variability across sites than seedling-based stands. Phenological patterns and fructification abundance also varied between the three clonal stands. These results suggest that clones with decreased reproductive ability can be chosen during the breeding process, lowering the dispersal risk while enhancing productivity. Thus, understanding the phenological behaviour of *E. globulus* is critical to more sustainable agroforestry by reducing the spread risks.

4.1 Introduction

Planted forests have reached nearly 300 million ha worldwide (FAO 2020) due to the ever-increasing demand for essential forest products such as wood, fibre and fuel. Exotic species occupy approximately 19% of these planted forests (Payn *et al*. 2015), some based on genetically improved clones selected for better growth and wood properties (Harfouche *et al*. 2012). However, despite their economic and social benefits, exotic plantations frequently raise concerns about their potential for naturalisation and dispersal, which could affect ecosystem functioning and biodiversity (Vilà *et al*. 2011; Liao *et al*. 2012; Thompson *et al*. 2014; Calviño-Cancela *et al*. 2016).

Eucalyptus globulus Labill. (Tasmanian blue gum) is one of the world's most widely planted forest species due to its excellent wood properties for the pulp and paper industry, rapid growth, and adaptability (Tomé *et al*. 2021). It is native to Australia, specifically Southern Victoria, Tasmania, and the Bass Strait Islands (Kirkpatrick 1975), with up to 13 geographical races and 20 subraces described (Dutkowski and Potts 1999). This species was introduced into Portugal around 1829 (Potts *et al*. 2004) and now covers approximately 850 000 ha, making it one of the country's dominant forest types (ICNF 2019). Since 1966, pulp companies in Portugal have been working on *E. globulus* genetic improvement programs based on local landrace populations and provenances introduced from Australia (Potts *et al*. 2004). These programs aim to improve tree growth, adaptability, and wood quality (Borralho *et al*. 1993). As a result, wood productivity has increased between 25 and 50% (Borralho *et al*. 2007), and trees are more resilient to biotic and abiotic stresses (Correia *et al*. 2014). However, reforestation with genetically improved trees has been mainly carried out on land managed by pulp companies, specifically through clonal plantations (Almeida *et al*. 2005), and many eucalypt plantations in Portugal are still based on unimproved seeds.

The phenological development of *E. globulus* takes about one year, from flower bud initiation to seed set, with trees producing flowers at four years old (Potts and Gore 1995; Jordan *et al*. 1999). Flower buds form from the current season's shoots, and their maturation result in the formation of flowers that are fully receptive to bird and insect pollination, particularly bees (House 1997; Hingston and Potts 1998). Eucalypt flowers are bisexual, but the breeding system is a mixed mating system with preferential outcrossing (Eldridge *et al*. 1993), with flowers potentially pollinated by many male parents, mostly from nearby trees with synchronised flowering (Jacobs 1979). Outcrossing is potentiated by protandry mechanisms, which cause the stigma to become fully receptive a few days after the flower's pollen matures and sheds (Jacobs 1979). Fruits are woody capsules with triangular valves on top (Eldridge *et al*. 1993) that change colour from green to brown as they mature over several months (Jacobs 1979). *Eucalyptus globulus* is a serotinous species that stores capsules for several years in the canopy while attached to tree branches, forming a canopy seed bank (Lamont *et al*. 1991) critical for seedling recruitment and dispersal events (dos Santos *et al*. 2015).

Temperature and moisture significantly impact eucalypt flowering (Eldrige *et al*. 1993), which is especially relevant as climate change affects air temperature and precipitation patterns (IPCC 2021). Furthermore, as reproductive structures require favourable environmental conditions, the reproductive phase is extremely vulnerable to climate change (Numata *et al*. 2022), which affects the timing of phenological events (Piao *et al*. 2019).

Concerns have been raised about *E. globulus*'s ability to regenerate and establish outside planted areas and become invasive (Sanz-Elorza *et al*. 2001; Gassó *et al*. 2010; Gordon *et al*. 2012; Rejmánek and Richardson 2013; Marchante *et al*. 2014). However, while quantitative studies have shown that its dispersal capacity is limited (Calviño-Cancela and Rubido-Bará 2013; Larcombe *et al*. 2013; Fernandes *et al*. 2016), regeneration can be abundant, particularly after fire (Águas *et al*. 2014; Calviño-Cancela *et al*. 2018; Anjos *et al*. 2021). Even if the intrinsic invasive capacity of *E. globulus* is limited, the large areas occupied by this species in some parts of the world raise concerns. Furthermore, as areas planted with improved trees are expanding, it is critical to investigate the impact of tree breeding programs on *E. globulus* reproductive biology due to their eventual effects on regeneration and invasiveness capacity, to better understand how to handle propagules and protect ecosystems near plantations. This concern was raised by Águas *et al*. (2017), who suggests a higher potential for greater fertility in these improved trees. To address these issues, the aim of this study was to identify the influence of stand type (clonal and seedling-based trees) and climate (mesic and xeric conditions) on the timing of the phenophases and production of reproductive structures of *E. globulus*.

4.2 Material and Methods

4.2.1 Study sites

This study was conducted in two sites located in different climatic regions in Portugal: Coimbra (40º10'19.6" N, 8º33'58.3" W, 65 m altitude) and Pegões (38°39'29.16" N, 8°37'16.23" W, 85 m altitude) (Fig. 4.1). The Coimbra site is cooler and wetter than Pegões. Coimbra's mean annual maximum and minimum temperatures are 21.6 and 10.8 ºC, respectively, while in Pegões, it is 22.3 and 11.5 ºC, respectively.

Mean annual precipitation differed by 200 mm, with 872.9 mm and 672.5 mm in Coimbra and Pegões, respectively. According to Köppen-Geiger Climate Classification, Coimbra is classified as Csb, a warm-summer Mediterranean climate, while Pegões is classified as Csa, a hot-summer Mediterranean climate (IPMA 2021).

Figure 4.1 Study site location: (mesic site) and Pegões (xeric site).

Both sites have a seasonal wet winter/dry summer pattern, with summer months presenting total mean precipitation of 50.8 mm in Coimbra and 18.9 mm in Pegões. August is the hottest month at both sites, with a mean difference of almost 2.5 ºC (28.6 and 31.1 ºC, mean maximum temperature). As mentioned, winter precipitation (from December to February) is higher in Coimbra (315.7 compared to 250.8 mm), and January is the coldest month with 5.8 ºC mean minimum temperature registered for Coimbra and 6.7 ºC for Pegoes [historical data 1990 – 2018, CRU-TS 4.03 (Harris *et al*. 2014) downscaled with WorldClim2.1 (Fick and Hijmans 2017)]. Hereafter, Coimbra will be referred to as a mesic site and Pegões as a xeric site. Climatic conditions (monthly total precipitation and mean temperatures) during our study were obtained from the closest meteorological stations and are presented in Fig. 4.2.

Both mesic and xeric sites exhibited similar weather seasonality with higher precipitation and lower temperatures in autumn and winter (October to February) and low rainfall and higher air temperatures in summer (June to August). The xeric site had higher maximum temperatures throughout the year, with the highest values recorded in July 2020 (32.6 ºC in the xeric site and 31.4 ºC in the mesic site). The beginning of 2019 was particularly dry at both sites compared with 2020 and 2021. However, winter 2019 in the mesic site was marked with considerable precipitation, 835.2 mm between October 2019 and April 2020, while the xeric site registered 392.8 mm. In terms of temperature, the minimum monthly values were registered in the xeric site, with the lowest temperatures recorded in January months in both sites: 4.8, 6.4 and 3.5 ºC in the xeric site and 6.0, 7.2 and 5.5 ºC in the mesic site. There was almost no summer precipitation in the xeric site (2.0 mm), while in the mesic site, the total precipitation of the three summer months was 92.3 mm.

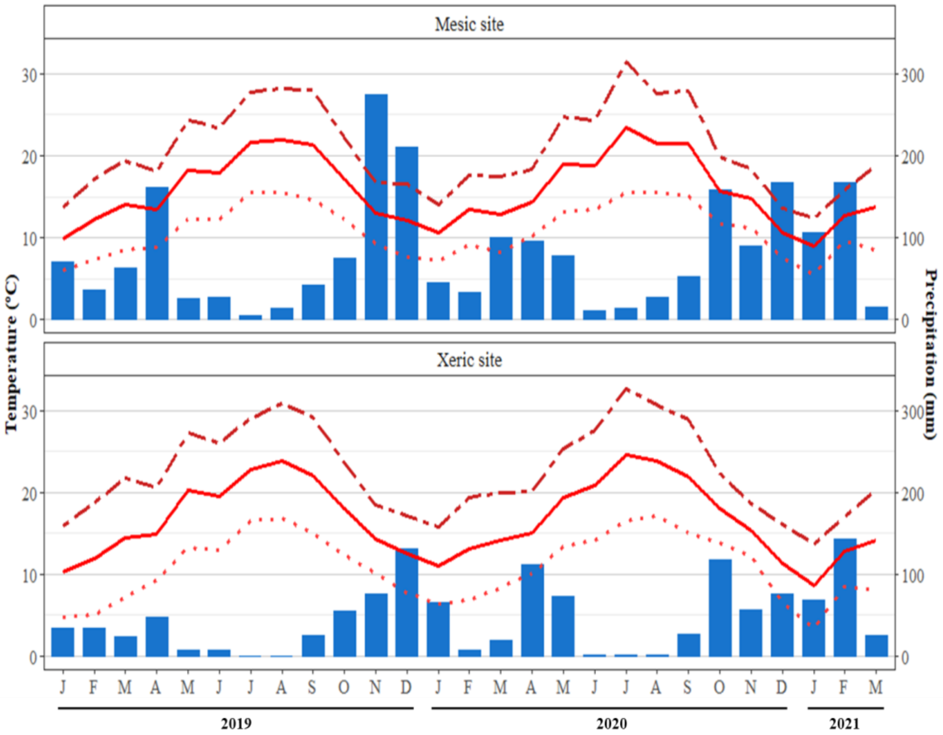


Figure 4.2 Climatic conditions for each study site. Monthly average temperature [◦C] (dotted line—minimum temperature; filled line—mean temperature; traced line—maximum temperature) and monthly total precipitation [mm] (bars) in the two study sites (mesic and xeric) from January 2019 to March 2021 by nearby meteorological stations.

4.2.2 Stand description

Both sites were under similar management regimes, with all stands located close to each other, with the same planting spacing (2 × 4 m) and having the same sun exposure. All stands are intensively managed with periodic understory clearings. The four different stands studied included one with plants grown from open-pollinated seedlings (uncontrolled crosses seedlings, SB) and three from clonal-origin, namely clones C1, C2 and C3. While SB stands have many different, non-improved genotypes, the clones are selected genotypes. Whereas clone C1 is a second-generation selection, a cross between a Portuguese landrace base parent and a "Strzelecki Range" (southern Australia origin) base parent, clones C2 and C3 are second-generation selections from unrelated crosses between Portuguese landrace base parents. Portuguese landrace mainly originated from southern and eastern Tasmania races (Freeman *et al*. 2007; Costa *et al*. 2017).

At the beginning of the study, stands at both sites have around six to nine years of age. The Perimeter at Breast Height (PBH) was measured in each tree (Table 4.1).

Table 4.1 *Eucalyptus globulus* stand description. Characterisation of the four stands (seedling-based, SB, and clones C1, C2 and C3): tree cut rotation (Rotation), plantation date and Perimeter at Breast Height (PBH) at the beginning of the study (mean ± SE, in cm).

Site	Stand	Rotation	Plantation date	PBH (cm)
Mesic	**SB**	1	2012	63.8 ± 3.4
	C1	1	2012	57.7 ± 2.5
	C2	1	2012	54.8 ± 2.3
	C3	1	2012	52.2 ± 1.8
Xeric	**SB**	3	2010	88.7 ± 8.8
	C1	1	2013	50.4 ± 3.0
	C2	1	2013	59.6 ± 4.1
	C3	1	2011	65.6 ± 2.6

4.2.3 Data Collection

At each site, 12 *E. globulus* trees from the four stands (clones C1, C2 and C3 and SB) were monitored every three weeks between 22 October 2019 and 17 March 2021. However, due to CoViD-19 legal restrictions, monitoring was interrupted on 6 March 2020 and resumed on 8 June 2020, resulting in four missed monitoring visits.

In each stand, trees were randomly selected along the plantation edge, where flowering is generally more abundant (Barbour *et al*. 2008). Each tree's genetic identity was confirmed in the clonal stands based on Simple Sequence Repeat (SSR) nuclear markers genotyping (*viz* Ribeiro *et al*. 2011). Selected trees were interspersed, and each tree was regularly monitored for different reproductive phenological stages, namely the presence of flowers and capsules, through binocular observation. According to their position on the branch, capsules were assigned to different years of maturation, with older capsules remaining more basal.

Since the phenological development of *E. globulus* take place between two different years (Goes 1977), capsules that matured in 2018 were classified as 2018 capsules even though the flower buds that originated them appeared in 2017. The same principle was applied to the capsules maturated in 2019, 2020, and 2021. Once 2018 and 2019 capsules were present at the start of the study, only the maturation of 2020 and 2021 capsules was recorded. Following Barbour *et al*. (2008), each reproductive stage was scored as a count on a logarithmic scale, with class 0 (absence of the stage), class 1 (1 to 10 reproductive structures), class 2 (11 to 100), and class 3 (≥ 101).

4.2.4 Data analysis

Statistical analysis was performed by transforming observation dates to julian days (jd), starting on 22 October 2019, corresponding to jd 295 and ending on 17 March 2021, corresponding to jd 806, by adding 365 days for each new year (Luedelling and Gassner 2012). Among the 12 studied trees of each stand in each site, only those that presented reproductive structures were considered to compare the initiation, end and duration of each phenological stage between sites through the Wilcoxon-Mann-Whitney test and among stands in each site with the Kruskal-Wallis test with the Bonferroni adjustment method to adjust *p* values to multiple comparisons. Due to CoViD-19 legal restrictions, the end of 2020 flowering was not completely recorded. However, considering that *E. globulus* flowering in Portugal occurs primarily between November and March (Goes 1977) and that flowers were in a declining phase on 6 March (the last record before the interruption), we assumed that the end of the flowering stage occurred on the next expected monitoring date (27 March) for statistical purposes.

The effect of stand type and site on each reproductive stage's intensity (scored in categorical classes) was evaluated using a Generalized Linear Mixed Model (GLMM) with Poisson and negative binomial families. For flower abundance, data of the two years of production were considered, with sampling year as a random factor, along with the monitored tree to account for these parameters' effect on the studied variables. Also, differences in reproductive stage intensity were evaluated between sites through the Wilcoxon-Mann-Whitney test and among stands in each site with the Kruskal-Wallis test with the Bonferroni adjustment method to adjust *p* values to multiple comparisons. Statistical analysis was performed using packages *agricolae* (Mendiburu 2021), *lme4* (Bates *et al*. 2015), *rstatix* (Kassambara 2021) and *stats* in R software version 4.2.2 (R Core Team 2022).

4.3 Results

4.3.1 General phenology pattern

4.3.1.2 The influence of genetic background

Clonal trees' phenological behaviour showed some differences comparing with seedling-based (SB) trees. Although no formal statistical analysis was performed to compare the percentage of trees showing reproductive structures in each stand, many trees did not produce any reproductive structure at both sites, particularly in clonal stands. For example, clonal C3 trees did not develop any reproductive structures in both sites throughout the monitoring period (or capsules matured in 2018 and 2019 were observed). Furthermore, clonal C2 trees did not produce flowers in the first year and exhibited the lowest frequency of the stands in the second year: seven out of twelve in the mesic site and five out of twelve in the xeric site. Moreover, in 2020, only seven SB trees and ten C1 trees produced reproductive structures in the xeric site (see Table 4.2). Also, it was observed in all stands that some trees produce flowers, but these do not transit to capsules. During the study, capsules from four seasons of maturation remained in the canopy at both locations, and only clonal C2 trees lost all their 2018 capsules in the mesic site (data not shown).

Differences in the initiation and duration of reproductive stages were observed when comparing clonal and SB trees within each site (Table 4.2). The onset of flowering (or anthesis) occurred in late autumn (end of November to early December), with capsules maturing throughout winter (from December to mid-February). In the mesic site, a delay in the onset of flowering of almost one month (end of January to early February, $p < 0.01$) was observed when comparing C1 and SB trees. In the second year, the same pattern of delayed emergence of flowers along with capsules in the clones was detected, but now for both C1 and C2 ($p < 0.001$), with clone C1 presenting again a later and shorter flowering ($p < 0.001$). In the xeric conditions, the phenological behaviour of SB and C1 trees was very similar in the first year, except for the flowering period, which was longer for the C1 clone ($p < 0.05$). In the second year, there was a much shorter flowering period, half of the duration of the other two stands ($p < 0.05$), in clone C2.

4.3.1.2 The influence of climate

The phenological behaviour of clonal stands differed more between the two sites than that of SB trees (Fig. 4.3). Seedling-based trees only had statistically significant site differences for the end of the flowering period, which ended earlier in the xeric site, although only in the first year ($p < 0.01$). Clone C1 showed the highest contrast in reproduction events between sites. For both years, flowering and capsule maturation occurred later ($p < 0.01$) in the mesic site. Clonal C2 trees produced reproductive structures only in the second year of monitoring, without statistically significant differences between flowers and capsule appearance.

Table 4.2 - Initiation and duration (mean ± SD) of reproductive phenological stages (flowers and capsules) for *E. globulus* seedling-based (SB) and clonal-based (clones C1, C2 and C3) stands during the first year (2019-2020) and the second year (2020-2021) in the mesic and xeric sites.

First-year

			Phenological stages				
			Flowering			**Fruit development**	
Site	**Stand**	**N**	**P**	**Initiation date**	**Duration (days)**	**P**	**Initiation date**
Mesic	**SB**	12	8	9 Jan±25^{a}	64±22^{a}	8	27 Feb±47^{a}
	C1	12	12	9 Feb±13^{b}	42±11^{b}	12	6 Mar±10^{a}
	C2	12	0	-	-	0	-
	C3	12	0	-	-	0	-
Xeric	**SB**	12	6	9 Jan±19^{a}	53±25^{a}	5	28 Jan±18^{a}
	C1	12	9	9 Jan±13^{a}	81±19^{b}	9	24 Jan±13^{a}
	C2	12	0	-	-	0	-
	C3	12	0	-	-	0	-

Second-year

			Phenological stages				
			Flowering			**Fruit development**	
Site	**Stand**	**N**	**P**	**Initiation date**	**Duration (days)**	**P**	**Initiation date**
Mesic	**SB**	12	12	26 Nov±32^{a}	102±37^{a}	12	25 Dec±16^{a}
	C1	12	8	13 Jan±15^{b}	51±19^{b}	8	12 Feb±0^{b}
	C2	12	7	14 Dec±17^{a}	62±29^{b}	7	2^{t} Jan±25^{c}
	C3	12	0	-	-	0	-
Xeric	**SB**	12	12	7 Dec±32^{a}	82±37^{a}	12	9 Jan±36^{ab}
	C1	12	12	1 Dec±28^{a}	86±44^{a}	12	20 Dec±30^{a}
	C2	12	4	25 Dec±11^{a}	32±12^{b}	2	12 Feb±0^{b}
	C3	12	0	-	-	0	-

Different letters indicate significant differences between initiation and duration among different stands in each site. N represents the total number of trees monitored; P represents the number of trees that developed a specific stage. The absence of phenological stages is marked with “-”.

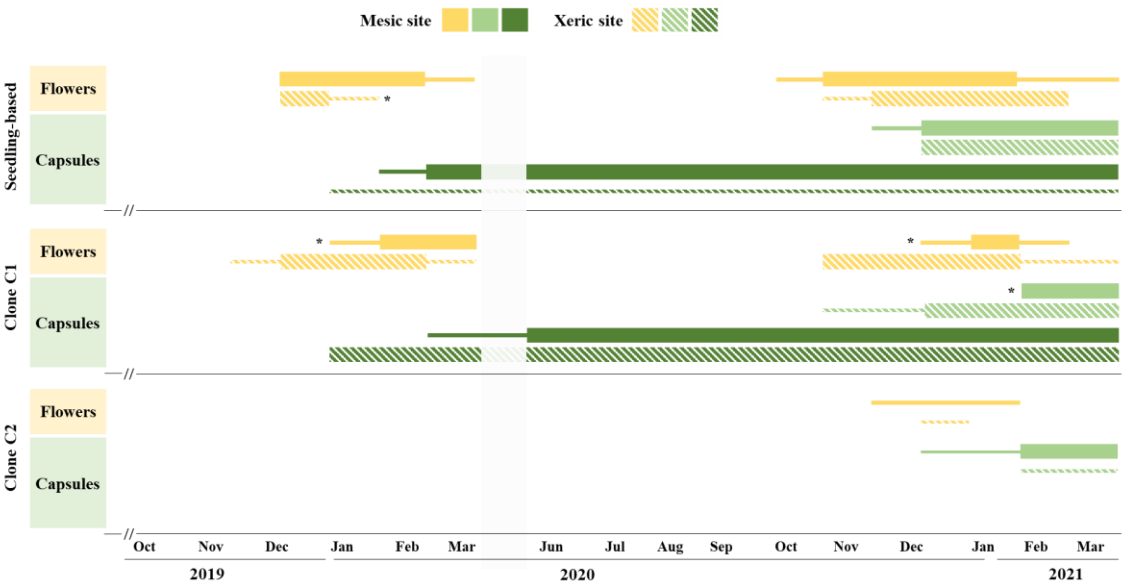


Figure 4.3 Phenophase diagram. Comparison of flowers and capsules from seedling-based and clonal-based (C1 and C2) *E. globulus* trees in the mesic and xeric sites from October 2019 to March 2021. White shaded bars represent the interruption due to CoViD-19 legal restrictions. Clone C3 did not exhibit any phenological structure during the monitored period, so data are not shown. Frequencies are indicated by lines (when 25–50% of the studied trees presented phenological structures) and filled bars (when over 50% of the studied trees presented phenological structures). Results for the mesic site are displayed in full lines and bars, while results for the xeric site are displayed in striped lines and bars. Significant differences between sites are denoted with "*" based on data presented in Supplementary Table S4.1.

4.3.2 Abundance of reproductive structures

The abundance of flowers and capsules was affected by genetic background, site and its interaction. The generalized linear mixed model showed that SB trees produced higher amounts of all reproductive structures than clonal stands, whereas, between clones, clone C1 presented a higher abundance of structures than C2 trees (Table 4.3).

In the first year of monitoring, the abundance of reproductive structures in the mesic site was similar between SB and clonal C1 trees. However, a higher abundance of flowers in SB trees was observed on 20 January compared with C1 trees, and a higher abundance of capsules was observed in SB trees on 20 February. Nonetheless, the number of capsules produced by SB and clone C1 was similar. In the second year, SB trees had a greater abundance for all phenological structures, whereas clonal C1 and C2 trees did not show differences in most of the monitoring period (Fig. 4.4).

On the contrary, under xeric conditions, during the first year of monitoring, clonal C1 trees produced more flowers and capsules, reaching a pick of flowering on 20 January 2020, while in the second year, SB and clonal C1 trees produced similar values, with clone C2 produced fewer reproductive structures.

Between studied sites, clonal C1 trees produced more structures under xeric conditions, except for the 2020 capsule' production; when producing, clone C2 presented more structures in the mesic site.

Table 4.3 Generalized linear mixed model (GLMM) using Stand (Seedling-based, Clone C1or Clone C2), Site (Mesic or Xeric) and their interaction to model *E. globulus* flower and 2020 and 2021 capsule production. Clone C3 did not exhibit any phenological structure during the monitored period, so data are not shown.

		Estimate	**Std. Error**	**z value**	**Pr (> |z|)**
Flower	(Intercept)	-0.628	0.13	-4.800	***
	Clone C1	-0.833	0.19	-4.308	***
	Clone C2	-1.656	0.25	-6.724	***
	Xeric site	-0.477	0.18	-2.673	**
	Clone C1 * Xeric site	1.221	0.26	4.613	***
	Clone C2 * Xeric site	-0.898	0.50	-1.791	ns
2020 capsules	(Intercept)	-0.227	0.14	-1.577	ns
	Clone C1	0.133	0.09	1.387	ns
	Clone C2	NA	NA	NA	NA
	Xeric site	-0.649	0.12	-5.450	***
	Clone C1 * Xeric site	0.729	0.15	4.911	***
	Clone C2 * Xeric site	NA	NA	NA	NA
2021 capsules	(Intercept)	-0.415	0.15	-2.702	**
	Clone C1	-1.303	0.30	-5.948	***
	Clone C2	-1.836	0.26	-5.120	***
	Xeric site	-0.053	0.20	-0.267	ns
	Clone C1 * Xeric site	1.848	0.36	5.083	***
	Clone C2 * Xeric site	-1.804	0.59	-3.059	**

Coefficients of the model, standard errors, the z statistic and the associated probabilities are presented.
ns = non-significant, * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001). NA – not applicable data

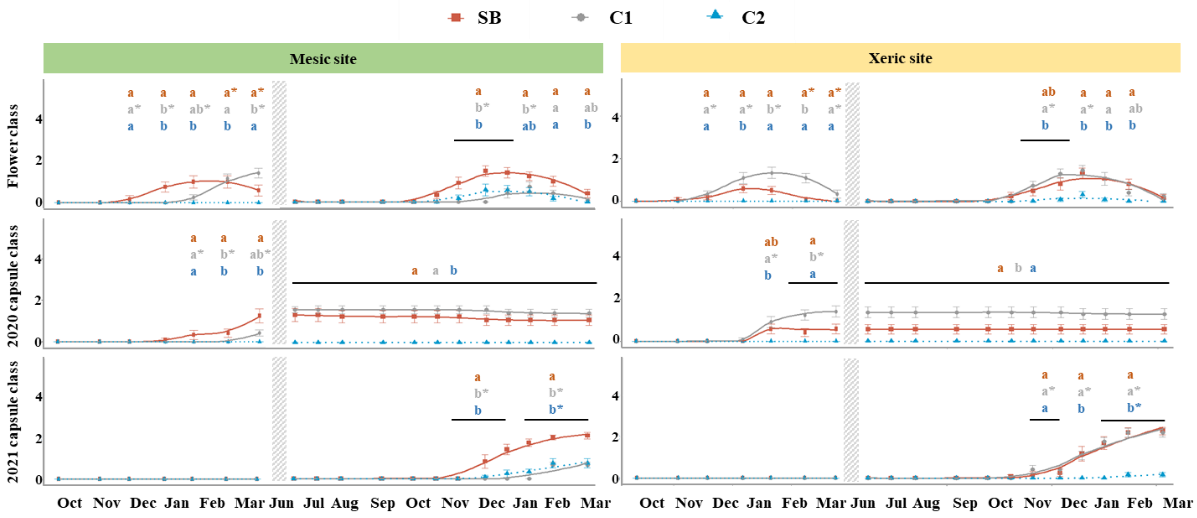


Figure 4.4 Abundance of reproductive structures. Comparison of the abundance of flowers, and 2020 and 2021 capsules from seedling-based (SB) and clonal-based (C1 and C2) *E. globulus* stands in the mesic and xeric sites from October 2019 to March 2021. Vertical shaded bars represent the interruption due to CoViD-19 legal restrictions. Clone C3 did not exhibit any phenological structure during the monitored period, so data are not shown. Different letters indicate significant differences regarding tree stand within each site; "*" indicate differences between study sites for each phenological class in each tree stand. Horizontal black lines represent the same pattern of differences within sampling dates.

4.4 Discussion

Eucalyptus globulus genetically improved clonal-based stands showed clear differences in the onset, persistence and abundance of reproductive phenological structures compared with unimproved plantations.

In Portugal (likewise many other countries), many eucalypt plantations rely on advanced generation genetically improved clonal forestry. These stands are more vigorous and uniform than their unimproved seedling-based (SB) counterparts. However, these clonal-based stands appear to have less abundant flowering, a slightly delayed onset, and a shorter duration, resulting in lower capsule production. These differences suggest that clonal-based forestry would have lower reproductive fitness, though these differences are likely clone-specific: while clone C3, one of Portugal's most commonly deployed materials, failed to produce reproductive structures in both study sites and years, clone C1 outperformed unimproved trees in the drier environment. On the other hand, clonal C2 trees flowered at the same time as SB trees only in the second year of monitoring, showing the shortest flowering period.

Indeed, it seems to occur a specific behaviour for each tree as well as an inter-annual variation as already described for *E. regnans* (Moncur and Boland 2000).

Precocity and phenology are strongly genetically controlled (e.g. Potts *et al*. 2001; Spencer *et al*. 2020), which could explain the flowering differences observed between clones C1 and C2. A longer flowering period was recorded for C2 trees under colder and wetter conditions and a particularly high inter-site variability for C1 trees, reflecting a delay in the flowering period of more than 40 days in the mesic site. This could be explained by the clone origins: clone C2 is derived from Southern Tasmanian base parents and adapted to colder conditions with no dry season. In contrast, clone C1 has Strzelecki sub-race ancestry, adapted to a warmer region in southern Australia (Australian Bureau of Meteorology 2021). Indeed, xeric conditions seemed to be associated with an earlier flowering initiation in clonal C1 trees, ultimately triggering earlier bud maturation and operculum shedding. In addition, following this information on clonal origins, it is also verified in terms of reproduction capacity that drier conditions favour the abundance of reproductive structures in clone C1, whereas clone C2 has greater success in mesic conditions. However, regardless of location characteristics, clonal trees had lower reproductive output than SB trees.

The differences in flowering between some of the clonal- and the seedling-based stands or the short flowering period of clonal trees could result in poor synchrony between the two stand types. In the former, this would be expected to lead to higher self-pollination rates (*viz* Hardner and Potts 1995; Barbour *et al*. 2008), resulting in lower seed set (Horsley and Johnson 2007) and poorer viability and development of the progeny (Hardner and Potts 1995; Chaix *et al*. 2013; Faia *et al*. 2022). However, under drier conditions, differences in the timing and duration of phenological stages were less apparent. Temperature is known to influence *Eucalyptus* flowering behaviour (Barbour *et al*. 2006; Hudson *et al*. 2010; Suitor *et al*. 2010) with early flowering usually found in warmer sites (Birtchnell and Gibson 2006), explaining the more homogenous flowering pattern observed in the xeric site.

Observations of up to three-year-old capsules in both SB and clonal trees have also been described for *E. regnans* (Cremer 1965; Ashton 1975). Older capsules gradually decreased over time, especially in the xeric site (data not shown), most likely due to more rapid leaf fall and subsequent branch death, a well-known strategy for coping with a longer summer season (Li *et al*. 2008). Understanding the demography of capsule dehiscence and seed storage in the canopy and how it varies with stand and site conditions is critical to determining plantation regeneration potential, particularly after a fire (Anjos *et al*. 2021).

Due to its importance in the paper industry, increased planting of *E. globulus* will raise concerns about its spread, invasion, and adverse impacts on ecosystems. Therefore, planting sterile genotypes or clones with low seed production will be crucial to limit dispersal events and should be a criterion of choice when selecting a genotype to deploy. This claim is noteworthy because clones C1 and C2 have similar numbers of seeds and germination rates as SB trees (data not shown), indicating that a reduced output of capsules reduces the probability of dispersal. However, since our findings revealed large variability in the reproductive behaviour of clones, new plantings should be continuously monitored to ensure that trees produce less seeds, not posing a threat.

Unfortunately, pandemic constraints hampered the continuous monitoring of the phenological behaviour of the studied trees. Notwithstanding, results pointed out different reproductive behaviour, especially among clones, including an inter-annual variation and an extreme of non-production. Future long-term studies should consider these phenological differences between clonal and SB trees. They are important to better interpret reproduction output over time, specifically seed viability and germination, ultimately clarifying the potential dispersal risk of genetically improved clonal stands versus unimproved stands.

Conclusions

This study indicates a different reproductive behaviour of clones compared with unimproved *E. globulus* stands, which may significantly impact the dispersal dynamics of this exotic species, a concern often raised by conservationists (Brundu *et al*. 2020). Seedling-based (SB) stands produced more flowers and capsules and behaved more consistently across climatic conditions. On the contrary, clonal-based stands were more variable, presenting significant inter-annual differences, shorter flowering seasons, less abundant reproductive structures and higher phenological variability across sites relative to SB plantations. It is relevant to denote that one of the studied clones did not produce any reproductive structures nor present remaining capsules; considering the tree cutting cycles performed in Portugal (i.e., 10‑12 years), even if a later in age flowering could be occurring, it will avoid seed production. These results are consistent with the hypothesis that the broader genetic basis ascribed to SB stands may result in greater reproductive fitness across different climatic conditions, whereas clonal trees may be more sensitive and less plastic (Jump *et al*. 2009). A better understanding of genetically improved clones' phenological behaviour may reduce regeneration risks and naturalization from this species in introduced ranges, providing an effective tool for sustainable management.

References

Águas A, Ferreira A, Maia P, Fernandes PM, Roxo L, Keizer J, Silva JS, Rego FC, Moreira F (2014) Natural establishment of *Eucalyptus globulus* Labill. in burnt stands in Portugal. For Eco Man 323:47-56. https://doi.org/10.1016/j.foreco.2014.03.012

Águas A, Larcombe MJ, Matias H, Deus E, Potts BM, Rego FC, Silva JS (2017) Understanding the naturalization of *Eucalyptus globulus* in Portugal: A comparison with Australian plantations. Eur J For Res 136:433-446. https://doi.org/10.1007/s10342-017-1043-6

Almeida MH, Araújo C, Araújo JA, Costa e Silva F, Neves I, Paiva V, Santiago A, Ribeiro D (2005) Melhoramento genético do eucalipto: que impacto na realidade? In: Silva R, Páscoa F (eds) A Floresta e as gentes, 5° Congresso Florestal Nacional. Sociedade Portuguesa de Ciências Florestais, Viseu, pp 145-146

Anjos A, Fernandes P, Marques C, Borralho N, Valente C, Correia O, Máguas C, Chozas S (2021) Management and fire, a critical combination for *Eucalyptus globulus* dispersal. For Eco Man 490: 119086. https://doi.org/10.1016/j.foreco.2021.119086

Ashton DH (1975) The root and shoot development of *Eucalyptus regnans* F. Moell. Aust J Bot 23:867-87. https://doi.org/10.1071/BT9750867

Australian Bureau of Meteorology (2021) Map of climate zones of Australia. Website. [21 November 2021] URL: http://www.bom.gov.au/climate/how/newproducts/images/zones.shtml

Barbour RC, Potts BM, Vaillancourt RE, Tibbits WN (2006) Gene flow between introduced and native *Eucalyptus* species: Flowering asynchrony as a barrier to F1 hybridisation between exotic *E. nitens* and native Tasmanian Symphyomyrtus species. For Eco Man 226:9-21. https://doi.org/10.1016/j.foreco.2006.01.017

Barbour RC, Otahal Y, Vaillancourt RE, Potts BM (2008) Assessing the risk of pollen-mediated gene flow from exotic *Eucalyptus globulus* plantations into native eucalypt populations of Australian. Biol Conserv 141:896-907. https://doi.org/10.1016/j.biocon.2007.12.016

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw 67(1):1-48. https://doi.org/10.18637/jss.v067.i01

Birtchnell MJ, Gibson M (2006) Long-term flowering patterns of melliferous *Eucalyptus* (Myrtaceae) species. Aust J Bot 54:745-754. https://doi.org/10.1071/BT05160

Borralho NMG, Cotterill PP, Kanowski PJ (1993) Breeding objectives for pulp production of *Eucalyptus globulus* under different industrial cost structures. Can J For Res 23(4):648-656. https://doi.org/10.1139/x93-085

Borralho NMG, Almeida MH, Potts BM (2007) O melhoramento do eucalipto em Portugal. In: Alves AM, Pereira JS, Silva JMN (eds) O eucaliptal em Portugal: Impactes ambientais e investigação científica. ISAPress, Lisbon, pp 61-110

Brundu G, Pauchard A, Pysěk P, Pergl J, Bindewald AM, Brunori A, Canavan S, Campagnaro T, Celesti-Grapow L, de Dechoum M de S, Dufour-Dror JM, Essl F, Flory SL, Genovesi P, Guarino F, Guangzhe L, Hulme PE, Jäger H, Kettle CJ, Krumm F, Langdon B, Lapin K, Lozano V, Le Roux JJ, Novoa A, Nuñez MA, Porté AJ, Silva JS, Schaffner U, Sitzia T, Tanner R, Tshidada N, Vítková M, Westergren M, Wilson JRU, Richardson DM (2020) Global guidelines for the sustainable use of non-native trees to prevent tree invasions and mitigate their negative impacts. NeoBiota 61:65-116. https://doi.org/10.3897/neobiota.61.58380

Calviño-Cancela M, Rubido-Bará M (2013) Invasive potential of *Eucalyptus globulus*: Seed dispersal, seedling recruitment and survival in habitats surrounding plantations. For Eco Man 305:129-137. https://doi.org/10.1016/j.foreco.2013.05.037

Calviño-Cancela M, Chas-Amil ML, García-Martínez ED, Touza J (2016) Wildfire risk associated with different vegetation types within and outside wildland-urban interfaces. For Eco Man 372:1-9. https://doi.org/10.1016/j.foreco.2016.04.002

Calviño-Cancela M, Lorenzo P, González L (2018) Fire increases *Eucalyptus globulus* seedling recruitment in forested habitats: Effects of litter, shade and burnt soil on seedling emergence and survival. For Eco Man 409:826-834. https://doi.org/10.1016/j.foreco.2017.12.018

Chaix G, Gerber SA, Razafimaharo V, Vigneron P, Verhaegen D, Serge H (2013) Gene flow estimation with microsatellites in a Malagasy seed orchard of *Eucalyptus grandis*. Theor Appl Genet 107:705-712. https://doi.org/10.1007/s00122-003-1294-0

Correia B, Pintó-Marijuan M, Neves L, Brossa R, Dias MC, Costa A, Castro BB, Araújo C, Santos C, Chaves MM, Pinto G (2014) Water stress and recovery in the performance of *two Eucalyptus globulus* clones: Physiological and biochemical profiles. Physiologia Plantarum 150:580-592. https://doi.org/10.1111/ppl.12110

Costa J, Vaillancourt RE, Steane DA, Jones RC, Marques C (2017) Microsatellite analysis of population structure in *Eucalyptus globulus*. Genome 60:770-777. https://doi.org/10.1139/gen-2016-0218

Cremer KW (1965) How Eucalypt fruits release their seed. Aust J Bot 13: 11-16. https://doi.org/10.1071/BT9650011 dos Santos P, Matias H, Deus E, Águas A, Silva JS (2015) Fire effects on capsules and encapsulated seeds from *Eucalyptus globulus* in Portugal. Plant Ecol 216:1611-1621. https://doi.org/10.1007/s11258-015-0544-y

Dutkowski GW, Potts BM (1999) Geographic patterns of genetic variation in *Eucalyptus globulus* ssp. *globulus* and a revised racial classification. Aust J Bot 47:237-263. https://doi.org/10.1071/BT97114

Eldridge K, Davidson J, Harwood C, Van Wyk G (1993) Eucalypt domestication and breeding. Claredon Press, Oxford

Faia J, Costa J, Araújo J, Borralho N, Marques C, Trindade H (2022) Impact of inbreeding on growth and development of young open-pollinated progeny of *Eucalyptus globulus*. iForest 15:356-362. https://doi.org/10.3832/ifor4012-015

FAO (2020) Global Forest Resources Assessment 2020: Main report. Rome. https://doi.org/0.4060/ca9825en

Fernandes P, Antunes C, Pinho P, Máguas C, Correia O (2016) Natural regeneration of *Pinus pinaster* and *Eucalyptus globulus* from plantation into adjacent natural habitats. For Eco Man 378:91-102. https://doi.org/10.1016/j.foreco.2016.07.027

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. Int J Climat 37:4302-4315. https://doi.org/10.1002/joc.5086

Freeman JS, Marques CMP, Carocha V, Borralho N, Potts BM, Vaillancourt RE (2007) Origins and diversity of the Portuguese landrace of *Eucalyptus globulus*. Ann For Sci 64:639-647. https://doi.org/10.1051/forest:2007042

Gassó N, Basnou C, Vilá M (2010) Predicting plant invaders in the Mediterranean through a weed risk assessment system. Biol Invasions 12:463-476. https://doi.org/10.1007/s10530-009-9451-2

Goes E (1977) Os eucaliptos: Ecologia, cultura, produções e rentabilidade. Lisboa, Portucel

Gordon DR, Flory SL, Cooper AL, Morris SK (2012) Assessing the invasion risk of *Eucalyptus* in the United States using the Australian weed risk assessment. Int J For Res 1-7. https://doi.org/10.1155/2012/203768

Hardner CM, Potts BM (1995) Inbreeding depression and changes in variation after selfing in *Eucalyptus globulus* spp. *globulus*. Silvae Genetica 44:46-54

Harfouche A, Meilan R, Kirst M, Morgante M, Boerjan W, Sabatti M, Scarascia Mugnozza G (2012) Accelerating the domestication of forest trees in a changing world. Trends Plant Sci 17:64-72. https://doi.org/10.1016/j.tplants.2011.11.005

Harris I, Jones PD, Osborn TJ, Lister DH (2014) Updated high-resolution grids of monthly climatic observations - The CRU TS3.10 Dataset. Int J Climat 34:623-642. https://doi.org/10.1002/joc.3711

Hingston AB, Potts BM (1998) Floral visitors of *Eucalyptus globulus* subsp. *globulus* in eastern Tasmania. Tasforests 10:125-140

Horsley T, Johnson S (2007) Is *Eucalyptus* cryptically self-incompatible? Annals Bot 100:1373-1378. https://doi.org/1373-8.10.1093/aob/mcm223

House SM (1997) Reproductive biology of eucalypts. In: Williams J, Woinarski J (eds) Eucalypt ecology: Individuals ecosystems. Cambridge University Press: Cambridge, pp 30-55

Hudson IL, Kim S, Keatley MR (2010) Climatic Influences on the Flowering Phenology of Four Eucalypts: A GAMLSS Approach. In: Hudson IL, Keatley MR (eds) Phenological research: Methods for environmental and climate change analysis. Dordrecht: Springer, pp 213-237. https://doi.org/10.1007/978-90-481-3335-2_10

ICNF (2019) IFN6 – Anexo técnico. 31 pp, versão 1.0 Instituto de Conservação da Natureza e das Florestas, Lisboa

IPCC (2021) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. https://doi.org/10.1017/9781009157896

IPMA (2021) Instituto Português do Mar e da Atmosfera. Website. [21 October 2021] URL: https://www.ipma.pt/pt/oclima/ normais.clima/

Jacobs MR (1979) Eucalypts for planting. Forestry series. Food and Agriculture Organization of the United Nations, Rome, Italy

Jordan G, Potts BM, Wiltshire R (1999) Strong, independent quantitative genetic control of vegetative phase change and first flowering in *Eucalyptus globulus* spp. *globulus.* Heredity (Edinb.) 83:179-8

Jump AS, Marchant R, Peñuelas J (2009) Environmental change and the option value of genetic diversity. Trends Plant Sci 14:51-58. https://doi.org/10.1016/j.tplants.2008.10.002

Kassambara A (2021) rstatix: Pipe-friendly framework for basic statistical tests. R package version 0.7.0. CRAN.R-project.org/package=rstatix.

Kirkpatrick JB (1975) Natural distribution of *Eucalyptus globulus* Labill. Aust Geogr 13(1):22-35. https://doi.org/10.1080/00049187508702675

Lamont BB, LeMaitre DC, Cowling RM, Enright NJ (1991) Canopy seed storage in woody plants. Bot Rev 57:277-317. https://doi.org/10.1007/ BF02858770

Larcombe MJ, Silva JS, Vaillancourt RE, Potts BM (2013) Assessing the invasive potential of *Eucalyptus globulus* in Australia: Quantification of wildling establishment from plantations. Biol Invasions 15:2763-2781. https://doi.org/10.1007/s10530-013-0492-1

Li X, Blackman CJ, Rymer PD, Quintans D, Duursma RA, Choat B, Medlyn BE, Tissue DT (2008) Xylem embolism measured retrospectively is linked to canopy dieback in natural populations of *Eucalyptus piperita* following drought. Tree Physiol 38:1193-1199. https://doi.org/10.1093/treephys/tpy052

Liao C, Luo Y, Fang C, Chen J, Li B (2012) The effects of plantation practice on soil properties based on the comparison between natural and planted forests: A meta-analysis. Glob Ecol Biogeogr 21:318-327. https://doi.org/10.1111/j.1466-8238.2011.00690.x

Luedelling E, Gassner A (2012) Partial least squares regression for analysing walnut phenology in California. Agric For Meteorol 158:43-52. https://doi.org/10.1016/j.agrformet.2011.10.020

Marchante H, Morais M, Freitas H, Marchante E (2014) Guia prático para a identificação de plantas invasoras em Portugal. Imprensa da Universidade de Coimbra

Mendiburu F (2021) agricolae: Statistical procedures for agricultural research. R package version 1.3.-5. CRAN.R-project.org/package=agricolae

Moncur MW, Boland DJ (2000) Production of genetically improved *Eucalyptus nitens* seed for reforestation, Aust For 63(3):211-217. https://doi.org/10.1080/00049158.2000.10674833

Numata S, Yamaguchi K, Shimizu M, Sakurai G, Morimoto A, Alias N, Azman N, Hosaka T, Satake A (2022) Impacts of climate change on reproductive phenology in tropical rainforests of Southeast Asia. Commun Biol 5(1). https://doi.org/10.1038/s42003-022-03245-8

Payn T, Carnus JM, Freer-Smith P, Kimberley M, Kollert W, Liu S, Orazio C, Rodriguez L, Silva LN, Wingfield MJ (2015) Changes in planted forests and future global implications. For Eco Man 352:57-67. https://doi.org/10.1016/j.foreco.2015.06.021

Piao S, Liu Q, Chen A, Janssens IA, Fu Y, Dai J, Liu L, Lian X, Shen M, Zhu X (2019) Plant phenology and global climate change: Current progresses and challenges. Glob Change Biol 25(6):1922-1940. https://doi.org/10.1111/gcb.14619

Potts B, Gore P (1995) Reproductive biology and controlled pollination of *Eucalyptus* - A review. School of Plant Science, University of Tasmanian, Hobart, Tasmanian

Potts BM, Barbour RC, Hingston AB (2001) Genetic pollution from farm forestry using eucalypt species and hybrids. A report for the RIRDC/L&WA/FWPRDC - Joint Venture Agroforestry Program. Rural Industries Research and Development Corporation

Potts BM, Vaillancourt RE, Jordan GJ, Dutkowski GW, Costa e Silva J, McKinnon GE, Steane DA, Volker PW, Lopez GA, Apiolaza LA, Li Y, Marques C, Borralho NMG (2004) Exploration of the *Eucalyptus globulus* gene pool. In: Borralho NMG, Pereira JS, Marques C, Coutinho J, Madeira M, Tomé M (eds) *Eucalyptus* in a changing world: proceedings of the IUFRO conference Aveiro, 11-15 October 2004. RAIZ, Instituto de Investigação da Floresta e Papel pp 46-61

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rejmánek M, Richardson DM (2013) Trees and shrubs as invasive alien species - 2013 update of the global database. Divers Distrib 19:1093-1094. https://doi.org/10.1111/ddi.12075

Ribeiro MM, Sanchez L, Ribeiro C, Cunha F, Araújo J, Borralho NMG, Marques C (2011) A case study of *Eucalyptus globulus* fingerprinting for breeding. Annals For Sci 68:701-714. https://doi.org/10.1007/s13595-011-0087-x

Sanz-Elorza M, Dana ED, Sobrino E (2001) Checklist of invasive alien plants in Spain (Iberian Peninsula and Balearic Islands). Lazaroa 22:121-131

Spencer B, Mazanec R, Abadi A, Gibberd M, Zerihun A (2020) Flowering phenology in a *Eucalyptus loxophleba* seed orchard, heritability and genetic correlation with biomass production and cineole: breeding strategy implications. Sci Rep 10(1):15303. https://doi.org/10.1038/s41598-020-72346-3

Suitor S, Potts BM, Brown PH, Gracie AJ, Rix KD, Gore PL (2010) The impact of flower density and irrigation on capsule and seed set in *Eucalyptus globulus* seed orchards. New For 39:117-127. https://doi.org/10.1007/s11056-009-9159-2

Thompson I, Okabe K, Parrotta J, Brockerhoff E, Jactel H, Forrester D, Taki H (2014) Biodiversity and ecosystem services: Lessons from nature to improve management of planted forests for REDD-plus. Biodivers Conserv 23. https://doi.org/10.1007/s10531-014-0736-0

Tomé M, Almeida MH, Barreiro S, Branco MR, Deus E, Pinto G, Silva JS, Soares P, Rodríguez-Soalleiro R (2021) Opportunities and challenges of *Eucalyptus* plantations in Europe: The Iberian Peninsula experience. Eur J For Res 140:489-510. https://doi.org/10.1007/s10342-021-01358-z

Vilà M, Espinar JL, Hejda M, Hulme P, Jarošík V, Maron J, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: A meta-analysis of their effects on species, communities and ecosystems. Ecol Let 14:702-8. https://doi.org/10.1111/j.1461-248.2011.01628.x

Supporting Information

Table S4.1 Timing (Start and End) (in julian days, mean ± SD) of reproductive phenological stages (flowers and capsules) for *E. globulus* stands from seedling or clonal-based (Clone C1, Clone C2 and Clone C3) from 2020 and 2021 in the mesic and xeric sites. Letters indicate significant differences between sites through Wilcoxon-Mann-Whitney test (α > 0.05). The absence of phenological stages is marked with "-".

		Flowers				**Capsules**	
		2020		**2021**		**2020**	**2021**
Stand	**Site**	**Start**	**End**	**Start**	**End**	**Start**	**Start**
Seedling-based	**Mesic**	374±25 ^{a}	438±16 ^{a}	695±32 ^{a}	797±35 ^{a}	423±47 ^{a}	724±16 ^{a}
	Xeric	356±19 ^{a}	409±14 ^{b}	706±32 ^{a}	788±36 ^{a}	393±18 ^{a}	739±36 ^{a}
Clone C1	**Mesic**	405±13 ^{a}	447±13 ^{a}	743±15 ^{a}	794±21 ^{a}	430±0 ^{a}	773±0 ^{a}
	Xeric	356±13 ^{b}	437±11 ^{a}	700±28 ^{b}	786±31 ^{a}	389±13 ^{b}	719±30 ^{a}
Clone C2	**Mesic**	-	-	713±17 ^{a}	779±20 ^{a}	-	751±25 ^{a}
	Xeric	-	-	724±11 ^{a}	756±12 ^{a}	-	773±0 ^{a}
Clone C3	**Mesic**	-	-	-	-	-	-
	Xeric	-	-	-	-	-	-

Chapter 5

FROM CAPSULES TO GERMINATION: AN ASSESSMENT OF *EUCALYPTUS GLOBULUS* DISPERSAL MECHANISMS

Anjos A, Jesus JG, Marques C, Borralho N, Valente C, Araújo JA, Máguas C, Correia O, Clemente A, Chozas S (2024) From capsules to germination: an assessment of *Eucalyptus globulus* dispersal mechanisms. *In preparation*

Abstract

Seed dispersal capacity is critical for plant species to spread into new regions and colonise new ecosystems. However, it can also contribute to invasion events when exotic species are introduced by humans into new areas. Concerns regarding invasion events have been raised, particularly in large-scale fast-wood plantations, such as *Eucalyptus globulus*, a commercially important species widely planted in Mediterranean-type environments. This species was introduced into Portugal in the mid-nineteenth century and currently accounts for nearly a quarter of the country's forest area. This area is mainly occupied with plantations with trees under 12 years old (i.e., seedling-based trees) but also, dispersed in the landscape, there are very old isolated trees (seed-trees) with high reproductive output. However, new plantations with genetically improved trees are being carried out aiming to increase production and wood quality. Since maternal genome (*viz* provenance), age, and climatic conditions (*viz* site) affect seed production and ecology of *E. globulus*, it is critical to analyse the potential risk of dispersal of different seed provenances. This study analyses the capsule morphology, seed set, and germination of clonal- and seed-trees under two different climatic conditions. We found that provenance and site significantly influenced capsule weight; capsules were heaviest in wetter conditions and lightest in clonal trees. Seed-trees' capsules presented a lower number of seeds in spite of having higher germination, while clonal trees' capsules had similar seed number when compared to seedling-based trees. Hence, it is crucial to consider both the reproductive biology and the plantation location when deploying clonal trees. Additionally, addressing concerns related to the presence of seed-trees is vital for ensuring a more sustainable long-term management of this exotic species, regarding its dispersal.

5.1 Introduction

Species reproduction capacity, particularly seed production and germination, is crucial for plant expansion, allowing them to disperse and establish in new areas, being a key step in invasion events (Lonsdale 1999; Rejmánek *et al*. 2005; Pyšek and Richardson 2007; Dawson *et al*. 2009; Blackburn *et al*. 2011). Species are being introduced into new ecosystems, particularly economically important fast-growing species, such as *Eucalyptus globulus*, planted worldwide due to global pulp demand (Rejmánek and Richardson 2013). These introductions can lead to biological invasion events, which impact the environment, economy, and human health by altering ecosystems and reducing biodiversity (Donaldson *et al*. 2013).

Eucalyptus globulus Labill. (Tasmanian blue gum) is endemic to Tasmania, the Bass Strait Islands, and southeast Australia (Kirkpatrick 1975). Thirteen geographic races with inherent trait differentiation have been described (Dutkowski and Potts 1999). Since its introduction into Portugal in the middle of the 19th century (Alves *et al*. 2007), the planted area has grown exponentially, now accounting for 26% of the country's forested area (ca. 845 000 ha) and making it the most important hardwood plantation species (ICNF 2019). The majority of plantations consist of unimproved trees, intensively managed through 10- to 12-year tree-cutting cycles (up to three rotations) (Soares *et al*. 2007). Recently, individuals with improved characteristics are being implemented in intensive clonal plantations (Borralho *et al*. 1992; Almeida *et al*. 2005). Tree improvement programs aim to increase pulp yield, select desirable wood properties (e.g., cellulose content and optimum fibre length), and also improve resistance to climatic conditions and pests (Potts *et al*. 2004; 2011). As a result of these breeding efforts, wood productivity increased by 25 to 50% (Borralho *et al*. 2007; Rezende *et al*. 2014) and improved timber is already being commercialized.

In contrast to monoculture systems, isolated *E. globulus* trees are dispersed throughout the landscape, usually delimiting properties and located near roadsides. These seed-trees are left uncut, with scarce management interventions, leading to substantial canopy growth. Consequently, these trees have a high production and accumulation of capsules (pers. obs.), thereby rendering them a significant source of propagules with a greater influence in dispersal risk (Anjos *et al*. 2021).

In plantation, *E. globulus* reaches sexual maturity at three to four years of age (Jordan *et al*. 1999). Phenological development takes about one year from pollination to seed set and pollination is animal-mediated (mainly insects); after fertilization, the ovary swells and eventually dries to form a mature woody capsule, identified when it turns brown (Jacobs 1979; Eldridge *et al*. 1993). Although capsules fall throughout the year (Calviño-Cancela and Rubido-Bará 2013), many remain in the canopy for at least four years (Anjos *et al*. 2023), forming a canopy seed bank (Lamont *et al*. 1991). Small, non-dormant seeds are mostly dispersed within capsules, with no specialized dispersal mechanism (Kirkpatrick 1977). Capsules protect seeds from predators and fire (Pryor and Johnson 1981; dos Santos *et al*. 2015), and their morphology varies between *Eucalyptus* species and geographical sub-races (Kirkpatrick 1975; Williams and Brooker 1997; McGowen *et al*. 2004), being genetically controlled (Suitor *et al*. 2009; Hernandez *et al*. 2022).

Although most seeds fall within the first 15 m of the plantation boundaries (Calviño-Cancela and Rubido-Bará 2013), the distance that seeds reach is approximately the horizontal distance corresponding to twice of the mother tree's height (Cremer 1977), which increases with tree age (Anjos *et al*. 2021). In any case, introducing *E. globulus* into a large planted area raises concerns about dispersal beyond the plantation's boundaries. Research conducted in Portugal has considered this species as naturalized (Catry *et al*. 2015; Fernandes *et al*. 2016; Deus *et al*. 2019). Seedling establishment is highly enhanced in disturbed areas such as after vegetation removal (Fernandes *et al*. 2017; 2018), nearby roadsides (Catry *et al*. 2015) and after fire (Águas *et al*. 2014; Anjos *et al*. 2021; Silva *et al*. 2021).

Hence, better knowledge of factors influencing reproductive capacity is important to evaluate the risk of dispersal, especially in the current context of increasing planted areas with genetically improved trees that could change the already known phenological behaviour of the species. In this sense, Anjos *et al*. (2023) referred that clonal trees have lower capsule production and greater variability across climatic conditions when compared to unimproved trees. Nevertheless, little is known about how improving trees affects the properties of capsule and seed, as well as how this improvement affects germination on clonal stands and in seed-trees, given their significant function as a source of propagules (Anjos *et al*. 2021; 2022). Within this framework, the main objective of this study was to evaluate the impact of *E. globulus* improvement in capsule and seed characteristics as well as on germination potential. To accomplish this, we measured the weight of the capsules, the number of seeds *per* capsule, and germination rate in two distinct climatic conditions, aiming to compare them in clonal, unimproved tree stands and seed trees.

5.2 Material and Methods

5.2.1 Study sites

This study was conducted in two different climatic regions of Portugal, according to the Köppen-Geiger climate classification: Coimbra, classified as Csb (warm-summer Mediterranean climate), and Pegões, classified as Csa (hot-summer Mediterranean climate) (IPMA 2022), referred to as mesic and xeric sites, respectively (Fig. 5.1).

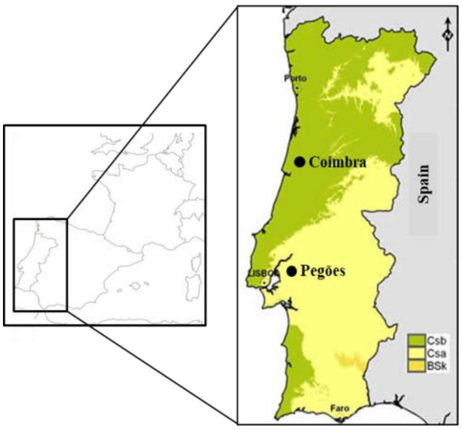


Figure 5.1 Location of study sites: trees were monitored in Coimbra (mesic site) and Pegões (xeric site) in Portugal mainland. Köppen-Geiger climate classification is represented: Csb (warm-summer Mediterranean climate), Csa (hot-summer Mediterranean climate) and Bsk (cold semi-arid climate). Adapted from IPMA 2022.

Both sites present a seasonal wet winter/dry summer pattern, with low rainfall in the summer months, especially in the xeric site. August is the hottest month at both sites, with a mean difference of almost 2.5 ºC (28.6 and 31.1 ºC, mean maximum temperature, for mesic and xeric sites, respectively). As mentioned, winter precipitation (from December to February) is higher in the mesic site (315.7 compared to 250.8 mm), and January is the coldest month with 5.8 ºC mean minimum temperature for the colder site and 6.7 ºC for the xeric site [historical data 1990-2018, CRU-TS 4.03 (Harris *et al*. 2014) downscaled with WorldClim2.1 (Fick and Hijmans 2017)]. Table 5.1 summarizes each site’s location and climatic description.

Table 5.1 Main characteristics of study sites.

		Mesic site	**Xeric site**
	Map reference	40º10’19.6” N, 8º33’58.3” W	38°39’29.16” N, 8°37’16.23” W
	Elevation (m)	65	85
	Köppen-Geiger Classification (*)	Csb	Csa
Historical data (mean values) (**)	**Annual min. and max. air temperatures (ºC)**	10.84 - 21.62	11.54 - 22.33
	Annual total precipitation (mm)	872.92	672.53
	Winter min. and summer max. air temperatures (ºC)	5.82 - 27.74	6.70 - 29.93
	Winter and Summer total precipitation (mm)	315.67 - 50.77	250.81 - 18.93

* Köppen-Geiger Classification (IPMA 2022): Csb - warm-summer Mediterranean climate; Csa - hot-summer Mediterranean climate | ** Historical data 1990-2018 (CRU-TS 4.03 (Harris *et al*. 2014) downscaled with WorldClim2.1 (Fick and Hijmans 2017)

5.2.2 Stands description

Three provenances of *E. globulus* stands were evaluated at each site: i) unimproved trees (hereafter, seedling-based), ii) isolated seed-trees with various genotypes resulting from seedling material found in the Portuguese landscape, and iii) second-generation clonal *E. globulus* stands. Clonal trees result from a cross between a Portuguese landrace base parent (mainly from races from southern and eastern Tasmania) and a "Strzelecki Range" base parent (from southern Australia) (Costa *et al*. 2017; Freeman *et al*. 2017). Seedling-based and clonal stands were located close to each other in the same property. Both stands were under similar management regimes, intensively managed with periodic understory clearings, with the same planting spacing (2 x 4 m) and the same sun exposure. At the start of the study, the stands at both sites were between six and nine years old. Isolated seed-trees of unknown age were dispersed across each property's surrounding areas (Table 5.2).

Table 5.2 *Eucalyptus globulus* stand description. Characterisation of the tree origins (seedling-based, clone and seed-tree): tree cut rotation (Rotation number), plantation date and diameter at breast height (DBH) at the beginning of the study (mean ± SE, in cm).

Site	Stand	Rotation	Plantation date	DBH (cm)
Mesic	**Seedling-based**	1	2012	20.3 ± 1.1
	Clone	1	2012	18.4 ± 0.8
	Seed-tree	1	Unknown	58.1 ± 10.0
Xeric	**Seedling-based**	3	2010	28.2 ± 2.8
	Clone	1	2013	16.1 ± 1.0
	Seed-tree	1	Unknown	71.0 ± 11.4

5.2.3 Data Collection

Sampling was carried out between June 2020 and March 2021. Twelve interspersed trees of average size (relative to the stand) of *E. globulus* stands (unimproved seedling-based and clonal trees) were selected along the plantation boundary, where flowers are more abundant (Barbour *et al*. 2008), in both mesic and xeric sites, along with seven older isolated seed-trees. A Simple Sequence Repeat (SSR) nuclear marker genotyping was performed to confirm the genetic identity of the clonal trees (Ribeiro *et al*. 2011).

Two 1.5 x 1.5 m plots (one in front and one on the side, without overlap) were set at the base of each trunk under the stand trees, a total of 24 plots *per* stand (Fig. 5.2a). In the case of seed-trees, given their proximity to private properties or the roadway, and the presence of dense vegetation, it was not possible to define 1.5 x 1.5 m plots, however one plot of 1 x 1 m was defined and monitored instead. All reproductive structures were removed from the plots at the beginning of the study (June 2020) (adapted from Calviño-Cancela and Rubido-Bará 2013). Plots were visited once a month and falling capsules were collected into paper bags.

5.2.3.1 Capsule and seed characterization

Capsules were stored in dry and at room temperature until processing. In the laboratory, capsules were assigned to different time since production based on colour, maturation, and abrasion (2021 = Y, 2020 = Y-1, 2019 = Y-2 and 2018 = Y-3) (Fig. 5.2b). Considering that *E. globulus* phenological development extends over two years (Goes 1977), capsules that mature in 2018 were classified as 2018 capsules (i.e., Y-3), even if the flower buds that produced them appeared in 2017. A similar approach was used to categorize capsules as Y, Y-1 and Y-2. For each capsule, it was determined whether it was dehisced (capsules that were already releasing material, operculum valve open) or full (no evidence of dehiscence, operculum valve closed). Some Y-1 capsules and all Y capsules were immature (green capsules, aborted) hence they were removed from the study. The diameter and height of each capsule were measured, and the shape of a cone was considered to provide a proxy for calculating the volume. Capsules were shaken to release all the seeds that were counted, and then weighted.

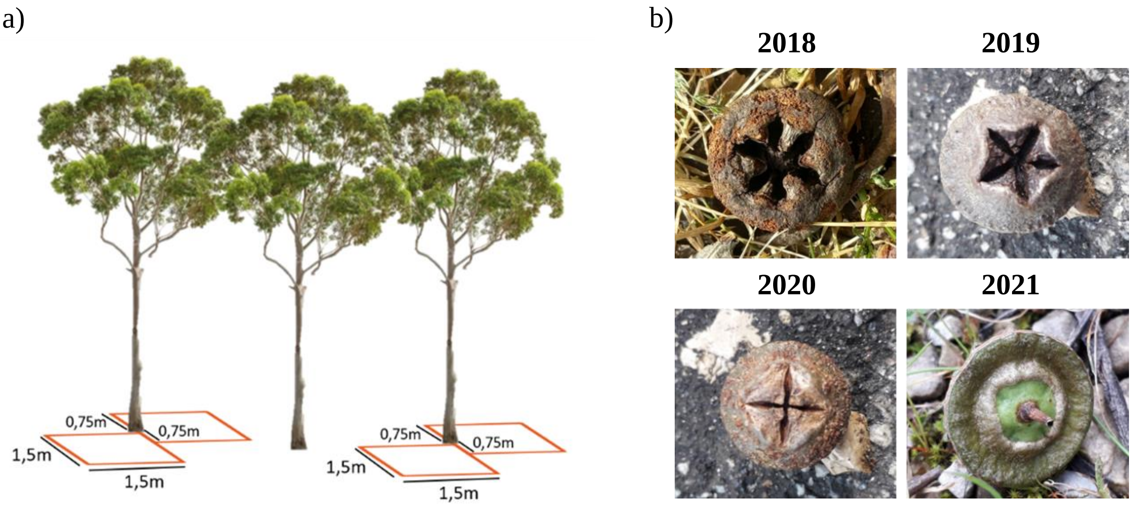


Figure 5.2 Sampling design implemented in the field consisting of a) 1.5 x 1.5 m plots and b) capsules from different years of production collected throughout the study (2018, 2019, 2020 and 2021).

5.2.3.2 Germination tests

Seeds were maintained in paper bags at room temperature (Jacobs 1979) until germination tests began. Seeds were grouped by site (Mesic or Xeric), tree provenance (seedling-based, seed-tree or clone) and time since production (Y-3, Y-2 or Y-1), and a total of 50 seeds were selected for germination tests, except for the combinations Mesic*seed-tree*Y-3 (35 seeds), Xeric*seed-tree*Y-3 (3 seeds) and Xeric*clone*Y-3 (3 seeds), since it was the maximum number of seeds collected. Seeds were placed in 9 cm diameter Petri dishes with a double layer of filter paper wetted with distilled water (25 seeds of each combination *per* Petri dish). Petri dishes were sealed with parafilm to ensure moist conditions and placed in the dark in a growth chamber (LMS 250/280 incubator) at 25 °C, the optimum temperature for *E. globulus* germination (Boland *et al*. 1980). New germinates were counted and then removed every two days during a month. Germination was considered when the seed presented the emergence of the radicle. Filter papers were frequently wetted with distilled water during the trial.

2.4 Data analysis

To determine the factors influencing capsule weight, a Generalized Linear Model (GLM) with gamma family was performed with the site (mesic or xeric), tree provenance (seedling-based, clone or seed-tree), and time since production (Y-3, Y-2 or Y-1) as explanatory variables. In this case, only full mature capsules were selected. As previously mentioned, the volume and weight of each capsule were assessed. Since capsule weight is more easily determined and both parameters showed a strong correlation (Spearman r = 0.84), this parameter was selected in all models.

To determine the factors influencing seed number, a GLM with binomial negative family was performed with the site (mesic or xeric), tree provenance (seedling-based, clone or seed-tree) and time since production (Y-3, Y-2 or Y-1). Once capsule dehiscence influenced seed number, comparison among site, stand type and year of production was made only with mature closed capsules.

Multiple pairwise comparisons of means and differences were performed through Kruskal-Wallis post-hoc tests with Bonferroni adjustment.

For the characterization of capsules, the correlation between the capsule weight and the seed number was evaluated through the Spearman correlation for each year of production.

Regarding germination tests, the final germination percentage (FGP) of seeds from different year production of all provenances for each site was calculated with the following formula:

$$\text{FGP (\%)} = n / N \times 100$$

where, n is number of germinated seeds and N is number of total seeds sown.

Data analysis was performed using packages *agricolae* (Mendiburu 2021), *lme4* (Bates 2015) and *stats* in R statistical software (R Core Team 2023).

5.3 Results

5.3.1 Capsule and seed characterization

Overall, we found that capsules from the mesic site were heavier and contained more seeds than those collected at the xeric site, with the highest values recorded for seedling-based trees (Tables 5.3 and 5.4). The studied clonal trees' capsules stored more seeds than those collected under seed-trees, despite its lighter weight. Furthermore, Y-2 capsules were heaviest and stored the highest number of seeds. Thus, all factors studied, i.e., site, provenance and time since production, had a significant impact on the development of capsules and seeds.

Table 5.3 Generalized Linear Model analysis of the effect of site (mesic or xeric), tree provenance (seedling-based, clone or seed-tree) and time since production (Y-3, Y-2 or Y-1) on *E. globulus* capsule weight. Significance p-value: ≤0.001 = ***; ≤0.01 = **; ≤0.05 = *; >0.05 = ns.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	1.853	0.027	67.851	***
(Mesic)				
Xeric	-0.093	0.020	-4.615	***
(Seedling-based)				
Clone	-0.400	0.024	-16.660	***
Seed-tree	-0.118	0.027	-4.302	***
(Y-3)				
Y-2	0.183	0.029	6.231	***
Y-1	0.002	0.030	0.071	

Table 5.4 Generalized Linear Model analysis of the effect of site (mesic or xeric), tree provenance (seedling-based, clone or seed-tree) and time since production (Y-3, Y-2 or Y-1) on *E. globulus* capsule seed number. Significance p-value: ≤0.001 = ***; ≤0.01 = **; ≤0.05 = *; >0.05 = ns.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	2.084	0.153	13.669	***
(Mesic)				
Xeric	-0.520	0.086	-6.033	***
(Seedling-based)				
Clone	-0.531	0.099	-5.342	***
Seed-tree	-1.304	0.123	-10.581	***
(Y-3)				
Y-2	0.848	0.161	5.267	***
Y-1	0.797	0.166	4.805	***

As previously mentioned, the heavier capsules were produced at both sites by seedling-based trees. With the exception of the Y-3 capsules, at the mesic site, where the weight of the seed-tree and clonal capsules were similar, the capsules collected from seed-trees had intermediate values. At the xeric site, capsules produced by the clonal trees were the lightest, and there were no differences in weight between the capsules produced by the seedling-based and seed-trees (Fig. 5.3).

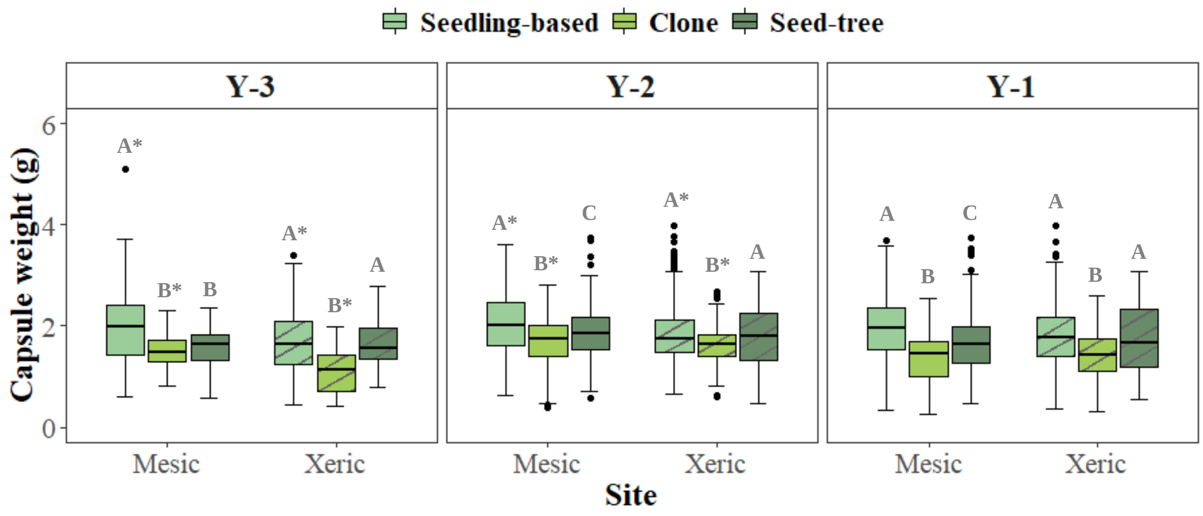


Figure 5.3 *Eucalyptus globulus* capsule weight (g) according to different times since production (Y-3, Y-2 or Y-1) in seedling-based and clonal-trees and seed-trees, in mesic and xeric sites. Different letters indicate significant differences regarding tree provenance within each site, calculated through the Kruskal-Wallis test with Bonferroni p-adjustment; "*" indicate significant differences between study sites for each tree provenance calculated with Wilcoxon-Mann-Whitney test ($\alpha < 0.05$).

Variations were found when comparing capsules from the same provenance between sites but limited to the oldest capsules (Y-3 and Y-2). In both cases, the mesic site produced the heavier capsules: Y-3 seedling-based tree capsules weighted 1.97 ± 0.05 and 1.71 ± 0.05 g (mean ± se) and Y-2 capsules weighted 2.05 ± 0.03 and 1.85 ± 0.02 g, for mesic and xeric sites respectively, while Y-3 clonal tree capsules weighted 1.53 ± 0.04 and 1.11 ± 0.11 g, and Y-2 capsules weighted 1.70 ± 0.04 and 1.62 ± 0.03 g.

In contrast to capsule weight, the more recent capsules showed greater differences regarding seed numbers (Fig. 5.4). For both sites, fewer seeds were counted in seed-tree capsules. Y-1 capsules collected under the clonal and seedling-based trees had a similar number of seeds, which was higher than for seed-trees, at both sites. In the mesic site, seed-tree produced 3.39 ± 1.8 seeds, while seedling-based and clonal trees produced 17 ± 4 and 12 ± 1, respectively; while in the xeric site, seed-tree capsules had 0.8 ± 0.34 seeds, and in seedling-based and clonal capsules 14 ± 3 and 6 ± 1 were counted. For Y-2 capsules, differences were only observed on the xeric site: higher number of seeds produced by seedling-based capsules (12 ± 1), while clonal and seed-trees produced 4 ± 1 and 2 ± 0.44 seeds, respectively.

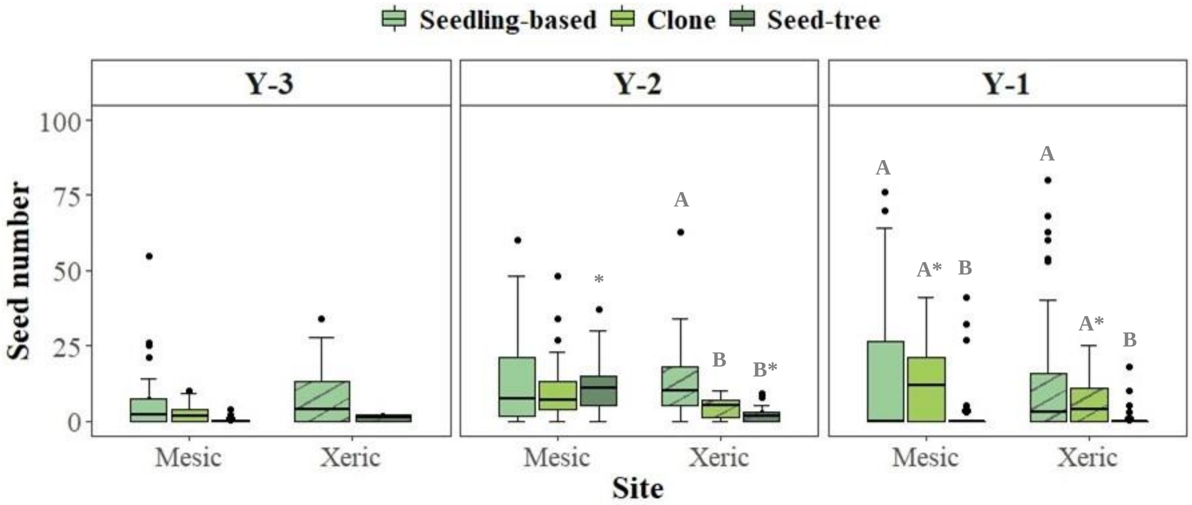


Figure 5.4 Capsule seed number according to different time since production (Y-3, Y-2 and Y-1) in *E. globulus* seedling-based, clonal and seed-tree. Note that there were no closed clonal Y-3 capsules in the xeric site. Different letters indicate significant differences regarding tree provenance within each site, calculated through the Kruskal-Wallis test with Bonferroni p-adjustment; "*" indicate significant differences between study sites for each tree provenance calculated with Wilcoxon-Mann-Whitney test ($\alpha < 0.05$).

Between sites, differences were observed in seed-trees Y-2 and in clonal Y-1 capsules, with higher values in the mesic site for both origins: 2 ± 1 compared to 2 ± 0.44 in the xeric site, in seed-trees and 12 ± 1 vs 6 ± 1 in clonal capsules.

For all years considered (*viz* time since production), a general positive correlation between seed number and capsule weight was observed, which decreases with age (Fig. 5.5).

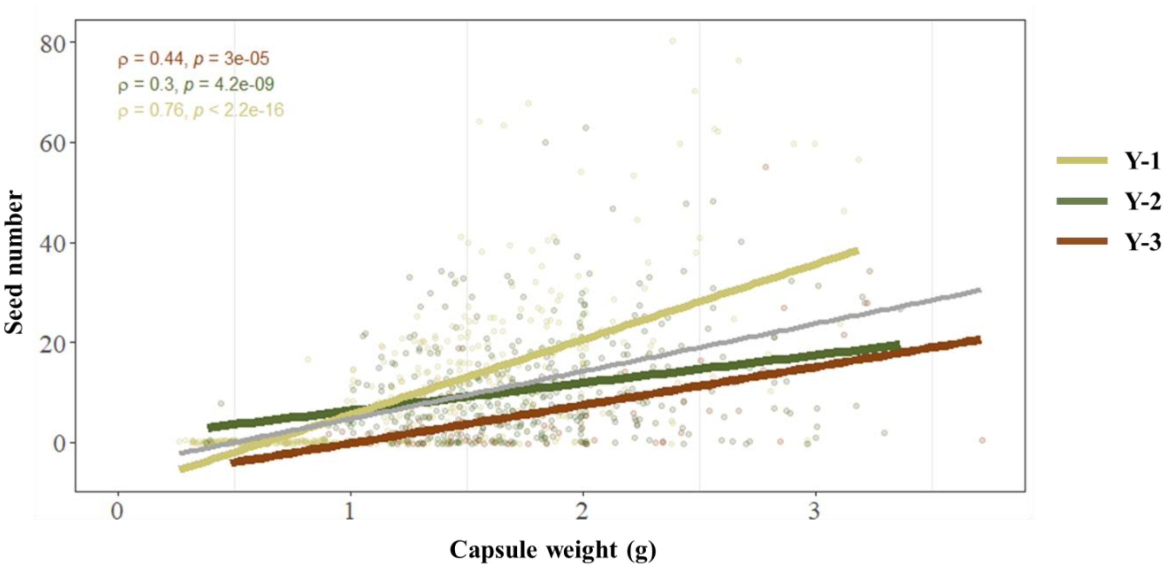


Figure 5.5 Correlation between capsule weight (g) and seed number for different time since production (Y-3, Y-2 and Y-1) *E. globulus* capsules, accounting for all stand provenances. Spearman R (rho) and p-value are presented in the figure. Correlations are represented with a linear fit lines and the black line represents the general linear fit among the two components regardless year of production, considering all stand types.

5.3.2 Germination rate

Overall, and for all provenances and in both sites, germination began on the second day after sowing and occurred during the first week (Fig. 5.6). At the mesic site, the germination of seeds produced by clonal- and seed-trees was similar with a higher percentage in recent seeds (96 and 94%, respectively). Older seeds had lower and similar germination: Y-2 and Y-3 seeds produced by clonal trees germinated 59 and 53%, respectively, while 48 and 49% were recorded for seed-trees. Germination decreased with age for seeds produced by seedling-based trees, a pattern observed for both studied sites (ca. 95, 63 and 34%, for Y-1, Y-2 and Y-3, respectively).

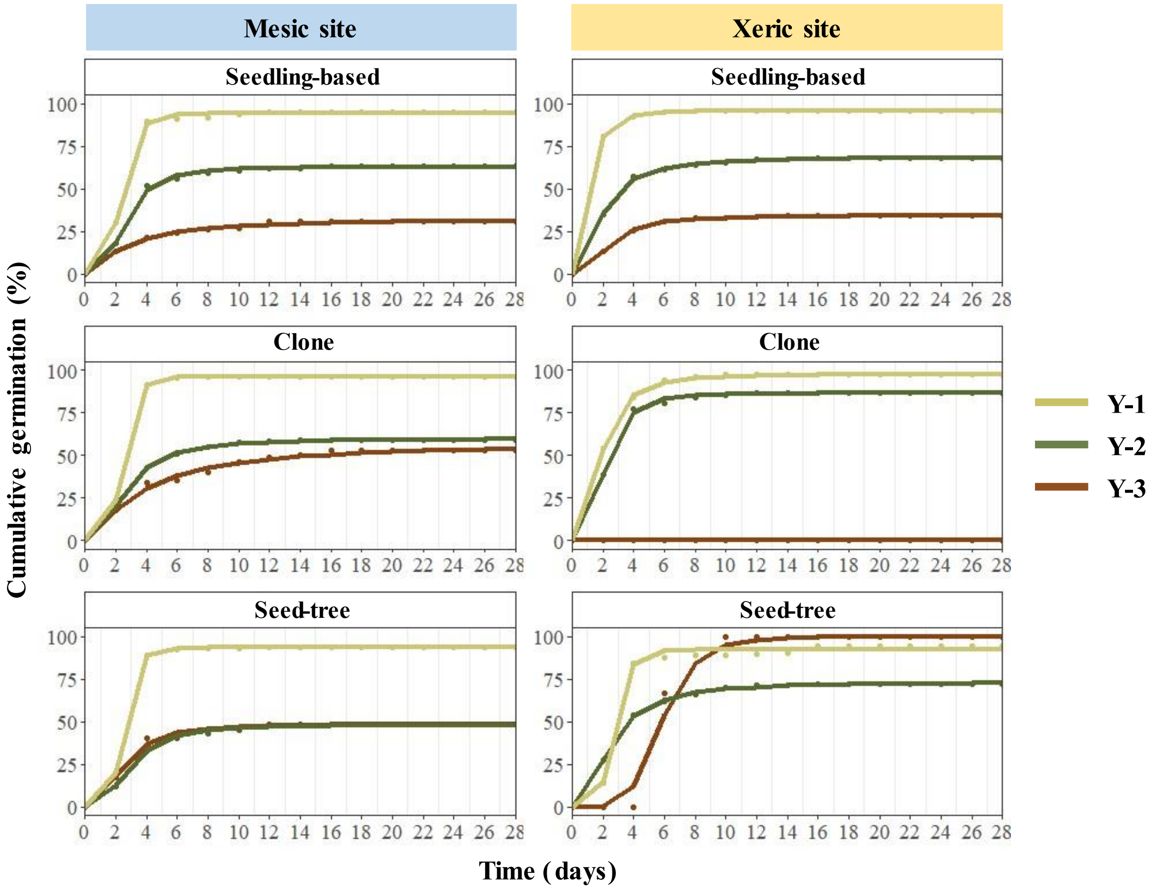


Figure 5.6 Cumulative percentage of germination of *E. globulus* seeds from different provenances (seedling-based, clone and seed-tree) including the different years of production (Y-3, Y-2 and Y-1). The time scale represents the days after sowing.

Also, in xeric site, seeds produced by clonal- and seed-trees showed greater values for Y-1 (ca. 97 and 94%, respectively) and Y-2 (87 and 72%), with Y-3 showing contrasting germination percentages: no germination for clonal and 100% for seeds produced by seed-trees.

When comparing mesic and xeric sites, recent seeds showed the greatest germination for all stands. For clonal trees, seeds from Y-2 had higher germination in xeric site (87 *versus* 59%), the same recorded for seed-trees for both Y-2 (72 and 48%) and Y-3 (100 and 48%).

5.4 Discussion

Our results indicate that different *Eucalyptus globulus* tree provenances present distinct capsule and seed traits. Also, tree age shows an important role in these characteristics that eventually affect the dispersal ability.

In fact, selecting sub-races for breeding programs results in changes in capsule and seed production (Jordan *et al*. 1993; Jones *et al*. 2002) since these traits are genetically controlled (Hernández *et al*. 2022). However, other variables such as moisture also affect capsule size (Ladiges and Ashton 1974), as we found in the mesic site, where capsules were larger and heavier.

Capsules collected from clonal trees were smaller and lighter when compared to seedling-based trees of similar age, which is probably related to their Strzelecki provenance, as suggested by McGowen *et al*. (2004). However, no significant differences were found in the number of seeds between both clonal and seedling-based trees, and germination rate of clonal seeds was high. In fact, it is known that large plantations composed with trees with the same genotype reduce the genetic variability of the stand and thus increase self-pollination (Hardner and Potts 1995; McGowen *et al*. 2004). Since in this study, the plantations followed were located in an experimental setting with numerous stands of different genotypes nearby, this could allow crossbreeding, explaining this similar number of seeds between seedling-based and clonal trees.

Additionally, even though the clone assessed was originated in hotter zones of Australia and had increased capsule production in the xeric site (Anjos *et al*. 2023), trees from the mesic site produced a greater number of seeds. This highlights a significant site effect, since higher water availability is associated with an elevated seed set (Suitor *et al*. 2010). However, this better adaptation to hotter conditions (Rix *et al*. 2015) was reinforced by higher values of germination in the xeric site for clonal seeds, unlike other studies, in which Strzelecki ranges maternal reduced germination (McGowen *et al*. 2004).

It is known that *Eucalyptus* large seed production enhances dispersal (Booth 2017), however, seed-trees produced fewer seeds than seedling-based trees, suggesting to rely more on production of capsules. Older eucalypt trees produce higher reproductive structures, since more energy is redirected into their production than younger trees, which invest in rapid growth (Birtchnell and Gibson 2006). As outcrossing is often higher in denser stands (Hardner *et al*. 1996) and inbreeding increases with age (Hardner and Potts 1995), seed-trees presented a lower seed set, a known post-zygotic barrier to self-pollination (Hardner and Potts 1995; McGowen *et al*. 2010). However, since outcrossing is more common in the upper canopy (Patterson *et al*. 2001), the fact that the capsule collection plots were located close to the seed-tree trunks may have underestimated the number of seeds collected, since capsules will fall far from the trunk in larger canopies (Cremer 1977). Nevertheless, germination is quite high, even for older seeds, which together with the higher production of capsules (pers. obs.) may explain its role as a propagule source (Anjos *et al*. 2021; 2022).

Since most pollination occurs within 200 m of nearby trees, it would be expected that clonal seeds presented lower germination, due to higher inbreeding (Byrne *et al*. 2008; Broadhurst 2013). However, more recent seeds showed similar high germination for all origins, while older seeds behave quite differently between the studied sites. Furthermore, in this study, higher mean germination percentages were obtained compared to other studies (Arán *et al*. 2013; dos Santos *et al*. 2015) since the age of the seeds was considered. Overall, the germination pattern was consistent with previous research (Arán *et al*. 2013; dos Santos *et al*. 2015), concentrated in the first week and consistent throughout all the years examined, proving the lack of dormancy of the seeds.

In fact, although capsules collected in the mesic site were heavier and presented higher seed set, seeds from the xeric site were heavier (data not shown), resulting in greater germination (Humara *et al*. 2001; Lopez *et al*. 2003) for clonal- and seed-trees. Indeed, Y-3 clonal seeds were the lightest (data not shown) not being able to germinate at all.

Finally, although capsules can dehisce while still attached to the tree branches, many remain closed and fall, releasing the seeds already in the ground (Free 1951). These seeds maintained the germination capacity, even in older capsules (i.e., capsules with at least four years), and may be relevant in disturbed areas for this species dispersal. It is important to highlight that the capsules were collected once a month, therefore the reproductive capacity of the seeds that are retained in the capsules is noteworthy; even in open capsules, seeds were found, since non-fertilized ovules are shed first while viable seeds are retained deep in the mature ovary (Eldridge *et al*. 1993), being released lately (dos Santos *et al*. 2015).

Long-term studies are then required to evaluate the reproductive biology of other clones. Nevertheless, these results indicate that using clones could reduce dispersal risk, since seedling-based trees not only exhibit greater capsule production and better adaptation to diverse environmental conditions (Anjos *et al*. 2023) but also generate more seeds per capsule, thereby enhancing potential new recruitment events.

Conclusions

Exotic species planting on a large scale may cause dispersal events that could result in invasion episodes, which poses a global threat to ecosystem stability (Blackburn *et al*. 2011).

Also, with increasing demand for forest products and concerns about sustainability, it is natural for advances such as tree improvement to emerge. In this sense, our findings reveal that the provenance of the *E. globulus* trees, the location of the plantation, as well as the tree age, influence seed output and germination capacity. Seed germination capacity is maintained regardless of time since production, being particularly relevant in clonal- and seed-trees. For this reason, reproductive biology of clonal trees must be considered when choosing and implementing improved plantations, including its location; while seed-trees are indeed a valuable seed source (*viz* quality over quantity), it is crucial to account with these old and isolated trees in management strategies.

References

Águas A, Ferreira A, Maia P, Fernandes PM, Roxo L, Keizer J, Silva JS, Rego FC, Moreira F (2014) Natural establishment of *Eucalyptus globulus* Labill. in burnt stands in Portugal. For Eco Man 323:47-56. https://doi.org/10.1016/j.foreco.2014.03.012

Almeida MH, Araújo C, Araújo JA, Costa e Silva F, Neves I, Paiva V, Santiago A, Ribeiro D (2005) Melhoramento genético do eucalipto: Que impacto na realidade? In: Silva R, Páscoa F (eds) A floresta e as gentes, 5° Congresso Florestal Nacional. Sociedade Portuguesa de Ciências Florestais, Viseu, pp 145-146

Alves AM, Pereira JS, Silva JN (2007) O eucaliptal em Portugal - Impactes ambientais e investigação científica. ISAPress, Lisbon

Anjos A, Fernandes P, Marques C, Borralho N, Valente C, Correia O, Máguas C and Chozas S (2021) Management and fire, a critical combination for *Eucalyptus globulus* dispersal. For Eco Man 490:119086. https://doi.org/10.1016/j.foreco. 2021.119086

Anjos A, Jesus JG, Marques C, Borralho N, Valente C, Correia O, Máguas C, Chozas S (2022) Climate and local factors influence *Eucalyptus globulus* establishment after off-season fires. New For 54:981-992. https://doi.org/10.1007/s11056-022-09950-w

Anjos A, Jesus JG, Marques C, Borralho N, Valente C, Araújo JA, Máguas C, Correia O, Chozas S (2023) Exploring reproductive biology in *Eucalyptus globulus* clones under distinct climatic conditions. Discover Agriculture 1(6). https://doi.org/10.1007/s44279-023-00006-x

Arán D, García-Duro J, Reyes O, Casal M (2013) Fire and invasive species: Modifications in the germination potential of *Acacia melanoxylon, Conyza canadensis* and *Eucalyptus globulus*. For Eco Man 302:7-13. https://doi.org/10.1016/j.foreco.2013.02.030

Barbour RC, Otahal Y, Vaillancourt RE, Potts BM (2008) Assessing the risk of pollen-mediated gene flow from exotic *Eucalyptus globulus* plantations into native eucalypt populations of Australia. Biol Conserv 141:896-907

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67(1):1-48. https://doi.org/10.18637/jss.v067.i01

Birtchnell MJ, Gibson M (2006) Long-term flowering patterns of melliferous *Eucalyptus* (Myrtaceae) species. Aust J Bot 54:745-754. https://doi.org/10.1071/BT05160

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26(7):333-339. https://doi/10.1016/j.tree.2011.03.023

Boland DJ, Brooker MIH, Turnbull JW (1980) *Eucalyptus* seed. CSIRO publishing, Canberra

Booth TH (2017) Going nowhere fast: a review of seed dispersal in eucalypts. Aust J Bot 65:401-410. https://doi.org/10.1071/BT17019

Borralho N, Cotterill PP, Kanowski PJ (1992) Genetic control of growth of *Eucalyptus globulus* in Portugal. II Efficiencies of early selection. Silvae Genet 41:70-77

Borralho NMG, Almeida MH, Potts BM (2007) O melhoramento do eucalipto em Portugal. In: Alves AM, Pereira JS, Silva JMN (eds.) O eucaliptal em Portugal - Impactes ambientais e investigação científica. ISAPress, Lisbon, pp 61-110

Broadhurst LM (2013) A genetic analysis of scattered Yellow Box trees (*Eucalyptus melliodora* A.Cunn. ex Schauer, Myrtaceae) and their restored cohorts. Biol Conserv 161:48-57. https://doi.org/10.1016/j.biocon.2013.02.016

Byrne M (2008) Phylogeny, diversity and evolution of eucalypts. In: Plant Genome: Biodiversity and Evolution. Volume 1, Part E, Phanerogams-Angiosperm (ed. ASA Sharma), Science Publishers, Enfield, pp 303-346

Calviño-Cancela M, Rubido-Bará M (2013) Invasive potential of *Eucalyptus globulus*: Seed dispersal, seedling recruitment and survival in habitats surrounding plantations. For Eco Man 305:129-137. https://doi.org/10.1016/j.foreco.2013.05.037

Catry FX, Moreira F, Deus E, Silva JS, Águas A (2015) Assessing the extent and the environmental drivers of *Eucalyptus globulus* wildling establishment in Portugal: results from a countrywide survey. Biol Invasions 17:3163-3181. https://doi.org/10.1007/s10530-015-0943-y

Costa J, Vaillancourt RE, Steane DA, Jones RC, Marques C (2017) Microsatellite analysis of population structure in *Eucalyptus globulus*. Genome 60:770-777. https://doi.org/10.1139/gen-2016-0218

Cremer KW (1977) Distance of seed dispersal in eucalypts estimated from seed weights. Aust For 7:225-228

Dawson W, Burslem DFRP, Hulme PE (2009) Factors explaining alien plant invasion success in a tropical ecosystem differ at each stage of invasion. J Ecol 97(4):657-665. https://doi.org/10.1111/j.1365-2745.2009.01519.x

Deus E, Silva JS, Larcombe MJ, Catry FX, Queirós L, dos Santos P, Matias H, Águas A, Rego FC (2019) Investigating the invasiveness of *Eucalyptus globulus* in Portugal: Site-scale drivers, reproductive capacity and dispersal potential. Biol Inv 21:2027-2044. https://doi.org/10.1007/s10530-019-01954-6

Donaldson JE, Hui C, Richardson DM, Robertson MP, Webber BL, Wilson JRU (2014) Invasion trajectory of alien trees: the role of introduction pathway and planting history. Glob Change Biol 20:1527-1537. https://doi.org/10.1111/gcb.12486

dos Santos P, Matias H, Deus E, Águas A, Silva JS (2015) Fire effects on capsules and encapsulated seeds from *Eucalyptus globulus* in Portugal. Plant Ecol 216:1611-1621. https://doi.org/10.1007/s11258-015-0544-y

Dutkowski GW, Potts BM (1999) Geographic patterns of genetic variation in *Eucalyptus globulus* ssp. *globulus* and a revised racial classification. Aust J Bot 47:237-263. https://doi.org/10.1071/BT97114

Eldridge K, Davidson J, Harwood C, Van Wyk G (1993) Eucalypt domestication and breeding. Claredon Press, Oxford

Fernandes P, Antunes C, Pinho P, Máguas C, Correia O (2016) Natural regeneration of *Pinus pinaster* and *Eucalyptus globulus* from plantation into adjacent natural habitats. For Eco Man 378:91-102. https://doi.org/10.1016/j.foreco.2016.07.027

Fernandes P, Máguas C, Correia O (2017) Combined effects of climate, habitat, and disturbance on seedling establishment of *Pinus pinaster* and *Eucalyptus globulus*. Plant Ecol 218(5):501-515. https://doi.org/ 10.1007/s11258-017-0706-1

Fernandes P, Máguas C, Correia O, González-Moreno P (2018) What drives *Eucalyptus globulus* natural establishment outside plantations? The relative importance of climate, plantation and site characteristics. Biol Invasions 20:1129-1146. https://doi.org/10.1007/s10530-017-1614-y

Fick SE, Hijmans RJ (2017) WorldClim 2: New 1km spatial resolution climate surfaces for global land areas. Int J Clim 37:4302-4315. https://doi.org/10.1002/joc.5086

Free RA (1951) Germination and emergence of eucalypt seed when sown at depth. Aust For 15(2):115-120. https://doi.org/10.1080/00049158.1951.10675810

Freeman JS, Marques CMP, Carocha V, Borralho N, Potts BM, Vaillancourt RE (2007) Origins and diversity of the Portuguese Landrace of *Eucalyptus globulus*. Annals For Sci 64:639-647. https://doi.org/10.1051/forest:2007042

Goes E (1977) Os eucaliptos: Ecologia, cultura, produções e rentabilidade. Lisboa: Portucel

Hardner CM, Potts BM (1995) Inbreeding depression and changes in variation after selfing in *Eucalyptus globulus* spp. *globulus*. Silvae Genet 44:46-54

Hardner CM, Vaillancourt RE, Potts BM (1996) Stand density influences outcrossing rate and growth of open-pollinated families of *Eucalyptus globulus*. Silvae Genet 45:226-228

Harris I, Jones PD, Osborn TJ, Lister DH (2014) Updated high-resolution grids of monthly climatic observations - the CRU TS3.10 Dataset. Int J Climatol 34:623-642. https://doi.org/10.1002/joc.3711

Hernández MA, Butler JB, Ammitzboll H, Weller JL, Vaillancourt RE, Potts BM (2022) Genetic control of the operculum and capsule morphology of *Eucalyptus globulus*. Ann Bot 130(1):97-108, https://doi.org/10.1093/aob/mcac072

Humara JM, Casares A, Majada J (2002) Effect of seed size and growing media water availability on early seedling growth in *Eucalyptus globulus*. For Eco Man 167:1-11. https://doi.org/10.1016/S0378-1127(01)00697-1

ICNF (2019) IFN6 – Anexo Técnico. [pdf], 31 pp, versão 1.0 Instituto da Conservação da Natureza e das Florestas, Lisboa

IPMA (2022) https://www.ipma.pt/pt/oclima/normais.clima/?print=true (consulted in May 2022)

Jacobs MR (1979) Eucalypts for planting. FAO, Rome

Jones RC, Steane DA, Potts BM and Vaillancourt RE (2002) Microsatellite and morphological analysis of *Eucalyptus globulus* populations. Can J For Res 32:59-66. https://doi.org/10.1139/x01-172

Jordan G, Potts BM, Kirkpatrick JB, Gardiner C (1993) Variation in the *Eucalyptus globulus* complex revisited. Aust J Bot 41:763-785. https://doi.org/10.1071/BT9930763

Jordan G, Potts BM, Wiltshire R (1999) Strong, independent quantitative genetic control of vegetative phase change and first flowering in *Eucalyptus globulus* ssp. *globulus*. Heredity (Edinb) 83:179-187. https://doi.org/10.1046/j.1365-2540.1999.00570.x

Kirkpatrick JB (1975) Natural distribution of *Eucalyptus globulus* Labill. Aust Geogr 13:22-35. https://doi.org/10.1080/00049187508702675

Kirkpatrick JB (1977) Eucalypt invasion in Southern California. Aust Geogr 13:387-393

Ladiges PY, Ashton DH. 1974. Variation in some central Victorian populations of *Eucalyptus viminalis* Labill. Aust J Bot 22:81–102

Lamont BB, LeMaitre DC, Cowling RM, Enright NJ (1991) Canopy seed storage in woody plants. Bot Rev 57:277-317

Lonsdale WM (1999) Global patterns of plant invasions and the concept of invasibility. Ecology, 80(5):1522-1536. https://doi.org/10.2307/176544

Lopez G, Potts B, Vaillancourt R, Apiolaza L (2003) Maternal and carryover effects on early growth of *Eucalyptus globulus*. Can J For Res 33. https://doi.org/10.1139/x03-132

Mendiburu F (2021) agricolae: Statistical procedures for agricultural research. R package version 1.3.-5. CRAN.R-project.org/package=agricolae

McGowen M, Potts B, Vaillancourt R, Gore P, Williams D, Pilbeam D (2004) The genetic control of sexual reproduction in *Eucalyptus globulus.* In: Borralho N, Pereira JS, Marques C, Coutinho J, Madeira M, Tomé M (eds) *Eucalyptus* in a changing world. Proc. of IUFRO Conf., Aveiro 11-15 October 2004

McGowen M, Vaillancourt R, Pilbeam D, Potts BM (2010) Sources of variation in self-incompatibility in the Australian forest tree, *Eucalyptus globulus*. Annals Bot 105(5):737-745. https://doi.org/10.1093/aob/mcq036

Patterson B, Vaillancourt RE, Potts BM (2001) Eucalypt seed collectors: beware of sampling seedlots from low in the canopy! Aust For 64(3):139-142. https://doi.org/10.1080/00049158.2001.10676178

Potts BM, Vaillancourt RE, Jordan GJ, Dutkowski GW, Costa e Silva J, McKinnon GE, Steane DA, Volker PW, Lopez GA, Apiolaza LA, Li Y, Marques C, Borralho NMG (2004) Exploration of the *Eucalyptus globulus* gene pool. In: Borralho NMG, Pereira JS, Marques C, Coutinho J, Madeira M, Tomé M (eds) *Eucalyptus* in a changing world: proceedings of the IUFRO conference Aveiro, 11-15 October 2004. RAIZ, Instituto de Investigação da Floresta e Papel pp 46-61

Potts B, Hamilton M, Blackburn D (2011) Genetics of Eucalypts: Traps and Opportunities. In: Walker J (ed) Developing a eucalypt resource: learning from Australia and elsewhere. Wood Technology Research Centre, University of Canterbury, New Zealand

Pryor LD, Johnson LAS (1981) *Eucalyptus*, the Universal Australian. In: Keast A (ed) Ecol Biogeograph Aust 1:499-536. The Hague, Junk

Pyšek P, Richardson D (2007) Traits associated with invasiveness in alien plants: Where do we stand? Biol Invasions 193. https://doi.org/10.1007/978-3-540-36920-2_7

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria

Rejmánek M, Richardson D, Higgins SI, Pitcairn M, Grotkopp E (2005) Ecology of invasive plants: State of the art. In: Mooney HA, Mc Neely JA, Neville L, Schei PJ, Waage J (eds) Invasive alien species: Searching for solutions. 104-162. Island Press,Washington, D.C

Rejmánek M, Richardson DM (2013) Trees and shrubs as invasive alien species - 2013 update of the global database. Divers Distrib 19:1093-1094. https://doi.org/10.1111/ddi.12075

Rezende GDSP, de Resende MDV, de Assis TF (2014) *Eucalyptus* Breeding for Clonal Forestry. In: Fenning T (ed) Challenges and Opportunities for the World's Forests in the 21st Century. Forestry Sciences, vol 81. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7076-8_16

Ribeiro MM, Sanchez L, Ribeiro C, Cunha F, Araújo J, Borralho NMG, Marques C (2011) A case study of *Eucalyptus globulus* fingerprinting for breeding. Ann For Sci 68:701-714. https://doi.org/10.1007/s13595-011-0087-x

Rix K, Gracie A, Potts B, Brown P, Gore P (2015) Genetic control of *Eucalyptus globulus* seed germination. Ann For Sci 72:457-467. https://doi.org/10.1007/s13595-014-0450-9

Silva JS, Nereu M, Pinho S, Queirós L, Jesús C, Deus E (2021) Post-fire demography, growth and control of *Eucalyptus globulus* wildlings. Forests 12:156. https://doi.org/10.3390/f12020156

Soares P, Tomé M, Pereira JS (2007) A produtividade do eucaliptal. In: Alves AM, Pereira JS, Silva JMN (eds) O eucaliptal em Portugal – Impactes ambientais e investigação científica. Lisbon, pp 27-60

Suitor S, Potts B, McGowen MH, Pilbeam DJ, Brown P, Gracie A, Gore P (2009) The relative contribution of the male and female to the variation in reproductive success in *Eucalyptus globulus*. Silvae Genet 58:129-138. https://doi.org/10.1515/sg-2009-0017

Suitor S, Potts BM, Brown PH, Gracie AJ, Rix KD, Gore PL (2010) The impact of flower density and irrigation on capsule and seed set in *Eucalyptus globulus* seed orchards. New For 39:117-127. https://doi.org/10.1007/s11056-009-9159-2

Williams JE, Brooker MIH (1997) Eucalypts: An introduction. In: Williams JE, Woinarski JCZ (eds) Eucalypt ecology: Individuals to ecosystems. Cambridge University Press, Cambridge, pp 1-15

Chapter 6

GENERAL DISCUSSION AND MAIN CONCLUSIONS

6. General Discussion

Introducing species into new regions can facilitate adaptation and potential dispersal. As a result, exploring novel niches with conditions similar to the native region creates a threat associated with the invasive potential in these introduced ecosystems. Although *E. globulus* is important for the world economy as a supply of forest products, its extensive planting raises concerns about its invasiveness (Cao *et al.* 2022). In this context, it is critical to identify the factors involved in the natural regeneration of this species in non-native areas to predict dispersal risk and provide more effective control measures. Several concerns and findings regarding this topic were addressed in the preceding chapters; this section presents a summary of the key results of this research about the main drivers that influence the natural establishment of *E. globulus* and provides recommendations for management.

Although it is known that forest fires promote the natural regeneration of *E. globulus* (Larcombe *et al.* 2013; Águas *et al.* 2014), this thesis shows how external factors significantly influence sapling establishment: pre-fire management strongly limits it, while the date and conditions following a fire, as well as the presence of older isolated seed-trees, enhance it (Chapters 2 and 3). Furthermore, significant recruitment episodes require the combination of several factors: fires followed by rainy episodes, plantations of a certain age that are not subjected to regular cut regimes, or the presence of isolated seed-trees, which due to their large size and height, increase density and the distance reached by saplings. On the other hand, in the absence of fire, recruitment of new individuals is extremely low, even under poor management conditions or nearby seed-trees, indicating a low spread capacity.

After a fire, seeds are released from capsules retained in canopies (dos Santos *et al.* 2015) and find favourable conditions for germination, such as bare soil and reduced inter-specific competition and allelopathic substances (Stoneman 1994). However, this research demonstrated that the date of the fire is crucial since dry conditions after a spring fire limit establishment, but fire occurrences before the rainy season stimulates recruitment (Chapter 2). In line with this, Silva *et al.* (2021) reported a mean of 322 000 saplings/ha and a maximum of 804 000 saplings/ha following the 2017 October fire event in Portugal, the highest density ever recorded in a non-native area.

Although the timing of the occurrence of a fire is uncontrolled due to meteorological factors, recurrent pre-fire management prevents significant recruitment events (Chapters 2 and 3). It was already known that periodic tree-cut rotation cycles avoid capsule production and seed maturation under no fire conditions by reducing propagule pressure and limiting the dispersal of wildlings (Fernandes *et al*. 2016), and this practice is also relevant to restraining recruitment even after a fire (Chapter 2). Plantations where trees are not periodically cut result in a higher accumulation of capsules in the canopy for at least three years (Chapter 4), increasing the risk of expansion (Chapter 3), since seeds maintain their viability during that period and can germinate when conditions are favourable (Chapter 5). Furthermore, the lack of management increases the growth of understory vegetation that stimulates fire spread and, as a result, potentially increases the burned area (Fernandes 2009). Consequently, frequent forest fires lead to rural abandonment and the development of unmanaged mixed plantations (Silva *et al*. 2011), maintaining the cycle that may result in frequent wildling regeneration potentiating *E. globulus* dispersal.

This study is especially significant because it includes, for the first time, isolated seed-trees as a potential source of propagules, emphasising their previously overlooked importance. Furthermore, accounting with their massive reproductive output, pre-fire management do not have as much of an impact on natural regeneration under seed-trees. In this case, the capsules accumulate in the soil throughout the year, and possibly when fire passes through, it destroys only the capsules in the upper layer, allowing the seeds in the bottom layers to germinate. Aside from increased recruitment, seed-trees provide more favourable microclimatic conditions for sapling establishment (Chapter 3). Post-fire resprouting in seed-tree branches can be especially determinant after spring fires to provide shade that reduces soil water evaporation and high soil-surface temperatures, increasing humidity and minimizing the impacts of water scarcity (Battaglia and Reid 1993; Stoneman 1994; Padilla and Pugnaire 2006); this will ultimately promote germination and establishment in such harsh circumstances. This statement is confirmed since seed-tree saplings withstand increasing aridity better than saplings growing nearby plantations (Chapter 3). Surprisingly, except for lack of management, local factors that influenced sapling establishment in seed-tree surrounding areas, namely stone and vegetation cover, altitude and aridity, had only a negative impact, reinforcing that the presence of these large trees is a great factor that enhances regeneration, pointing seed-trees as nurse plants (Cavieres *et al*. 2006), once their presence facilitates the development of saplings. These findings highlight the critical role of seed-trees as a source of wildlings and the need to consider these trees in management strategies.

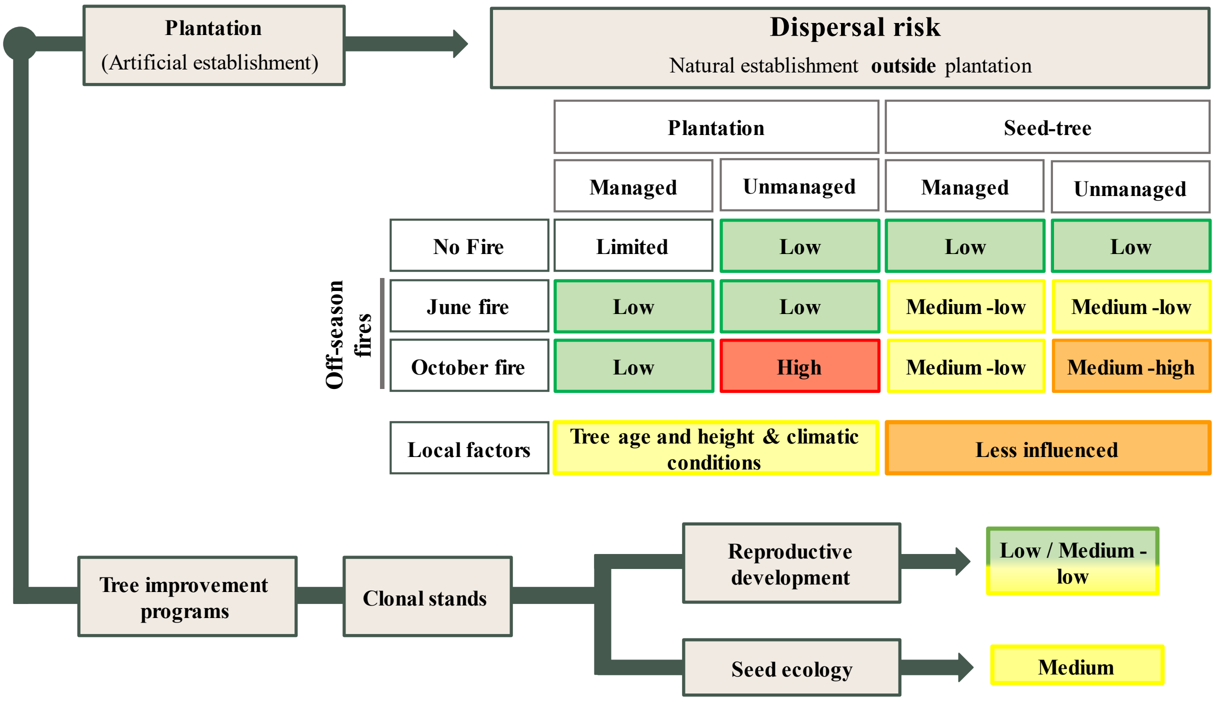
Despite this, saplings were mainly observed close to the plantation's limits or seed-trees, especially in the first 30 m, confirming the low dispersal capacity of *E. globulus*. Also, even after a fire, when conditions favour the germination and establishment of new individuals, local factors play a relevant role, supporting the species' limited ability to invade new areas.

However, a significant sapling survival was reported even two years following the fire events in areas with no post-fire management (Chapter 2). However, offspring density is expected to decrease (da Silva *et al*. 2011; Calviño-Cancela and Rubido-Bará 2013; Lorentz and Minogue 2015; Silva *et al*. 2021), most likely due to intra-specific competition (Adams *et al*. 1993), yielding similar results to Águas *et al*. (2014), who observed only 0.2 plants/m^2 (with a maximum of 4.6 plants/m^2) five years after a fire. In this regard, it is observed that there seem to be periods of species naturalization, with reproduction occurring at a short distance from the mother trees. However, climate change may dodge this scenario considering an extension of the fire season ascribed to different post-fire conditions. For example, the worst-case scenario was found after autumn fires in unmanaged plantations with the highest natural regeneration, opening the door to potential invasive events. Nonetheless, seed-trees' presence led to a constant source of wildlings since increased sapling cover was detected in all remaining combinations.

Given the potential risk of dispersal after fire events, clonal tree plantations should be preferred over seedling-based ones, since the ones that were studied produce fewer reproductive structures (Chapter 4) and less seeds per capsule (Chapter 5), which will limit the establishment and subsequent dispersal of wildlings and minimize concerns about *E. globulus* dispersal. Furthermore, within monoculture plantations of the same genotype trees, despite pollen being able of dispersing about 700 m (Costa *et al*. 2022; Faia *et al*. 2022), insects will not be capable to pollinate flowers of different genotypes, thereby enhancing inbreeding. Additionally, even when multiple clones are present, out-crossing becomes more difficult due to variations in flowering time and duration among trees (Chapter 4). However, climate change could resemble flowering periods, increasing pollination efficiency, so constant monitoring of these clones with lesser reproductive capacity will be required.

The widespread utilization of clones in planted forests could potentially limit reproductive capacity in certain scenarios, such as unmanaged or abandoned plantations, or even if one of these trees became a seed-tree. In this sense, this limitation on reproductive capacity may be seen as a key sustainable practice, particularly in mitigating the potential risk of natural regeneration outside plantation.

Overall, this thesis provides insights to the ongoing controversy surrounding *E. globulus* dispersal, especially in new scenarios (Fig. 6.1) by addressing the identified knowledge gaps and through its main findings.



		Plantation		**Seed-tree**	
		Managed	**Unmanaged**	**Managed**	**Unmanaged**
	No Fire	**Limited**	**Low**	**Low**	**Low**
Off-season fires	**June fire**	**Low**	**Low**	**Medium -low**	**Medium -low**
	October fire	**Low**	**High**	**Medium -low**	**Medium -high**
	Local factors	**Tree age and height & climatic conditions**		**Less influenced**	

Figure 6.1 Main outcomes from this thesis, considering the gaps in knowledge ascribed to *E. globulus* establishment and dispersal.

In a nutshell it is crucial to emphasize the low risk associated with *E. globulus* in several contexts: i) in the absence of a fire, ii) following a fire combined with pre-fire management and iii) after spring fires. However, the novel case of clonal stands implies particular caution due to the heterogeneity observed among clones. Even so, in contrast to seedling-based trees, clones displayed site-specific reproductive behaviour ascribed to a lower capsule production and seed set. However, under favourable conditions clones could establish new individuals, thereby creating opportunities for naturalization.

On the other hand, there are some scenarios that raise concerns, particularly those involving the presence of seed-trees. Given their huge reproductive output, when combined with fire occurrence and its capacity to mitigate harsh conditions, seed-trees promote the naturalization and could contribute to the expansion process. However, as mentioned previously, the worst-case scenario which poses the highest level of risk is the lack of management in plantations along with autumn fires, joining residence time and the highest propagule pressure. Even so, the largest sapling cover was found within the plantation, and a greater dispersal was restricted to 15 m beyond the plantation limits, confirming this species low dispersal capacity. Moreover, it is crucial to account with local drivers influence on post-fire natural regeneration, since saplings have shown high sensitivity to surrounding conditions. Therefore, considering these outcomes, managers could decide the best management strategies aiming to prevent dispersal events.

6.1 Management recommendations

Given the known critical role of high propagule pressure in an invasive process (Rejmánek *et al*. 2005), optimal management strategies must target this attribute, whether from large plantation areas or high reproductive output. As a result, adhering to best practices and environmental guidelines can help reduce the expansion risk.

Even though saplings establish mainly in the first 15 m around plantation edges and seed-trees, and their control after a fire is effective (Águas *et al*. 2014; Silva *et al*. 2021), control measures using mechanical tools in burned areas are recommended to mitigate any dispersal events, particularly in the surrounding areas of the isolated trees. Moreover, dispersal could be reduced by improving clone selection and silviculture. Firstly, the higher productivity of improved trees may result in a decrease of planted areas, which, in addition to minimizing the ecological implications of monoculture, also reduces the area suitable for dispersal. Also, deploying improved forests, besides enhancing forest management due to greater uniformity (Rezende *et al*. 2014), can combine reduced reproductive output with higher tree productivity. Alternatively, interspersing seedling-based plantations with reduced reproductive capacity clonal plantations could reduce seed production levels. Nevertheless, before adopting these improved trees, a phenological assessment is recommended to ensure lower seed output or even absence of production. In any case, short-rotation tree-cut cycles should be advised to reduce flower and seed production and eliminate wildlings.

6.2 Conclusions

Planted forests provide economic benefits and are a desirable alternative to exploiting native forests. However, special consideration must be given to the spread of exotic species, since introduced species originated from similar areas generally demonstrate strong adaption to new conditions and can cause significant damage in introduced areas. *Eucalyptus globulus* does not appear to be an invasive species in Portugal due to its poor spread capacity outside the plantations, even in the presence of disturbances such as fire. Moreover, despite its high propagule production, the species does not disperse beyond the limits of most plantations, making its naturalization *status* based on its large planted area. Additionally, lower sapling cover in burnt and unburnt managed areas indicates that lower residence time is crucial in lowering this species' natural regeneration and highlights for the positive effect of management even in a pre-fire scenario. On the other hand, seed-trees are of concern as they contribute greatly to species dispersal.

Regarding clonal trees, and because they vary in their reproductive biology, selecting sterile genotypes or with limited seed production can reduce dispersal risk. Additionally, short rotations minimize seed maturation and the number of years with substantial seed production. Even when employing low seed production trees, monitoring should be prioritized since climate change and adaptation to new conditions may increase dispersal events.

Finally, the most effective management strategy for dealing with dispersal events is prevention. Identifying the main factors responsible for the spread of an introduced species allows the implementation of efficient prevention techniques, early identification of recruitment events, and rapid actions to ultimately avoid an potential invasion. Together, these strategies will ultimately ensure the maintenance of productive planted forests due to their subsequent importance along with an effort to value biodiverse native forests.

References

Adams P, Beadle C, Mendham N, Smethurst PJ (2003) The impact of timing and duration of grass control on growth of a young *Eucalyptus globulus* Labill. plantation. New For 26:147-165. https://doi.org/10.1023/A:1024490707175

Águas A, Ferreira A, Maia P, Fernandes PM, Roxo L, Keizer J, Silva JS, Rego FC, Moreira F (2014) Natural establishment of *Eucalyptus globulus* Labill. in burnt stands in Portugal. For Eco Man 323:47-56. https://doi.org/10.1016/j.foreco.2014.03.012

Battaglia M, Reid JB (1993) The effect of microsite variation on seed germination and seedling survival of *Eucalyptus delegatensis*. Aust J Bot 41:169-81. https://doi.org/10.1071/BT9930169

Calviño-Cancela M, Rubido-Bará M (2013) Invasive potential of *Eucalyptus globulus*: Seed dispersal, seedling recruitment and survival in habitats surrounding plantations. For Eco Man 305:129-137. https://doi.org/10.1016/j.foreco.2013.05.037

Cao R, Gong X, Feng J, Yang R (2022) Niche and range dynamics of Tasmanian blue gum (*Eucalyptus globulus* Labill.), a globally cultivated invasive tree. Ecol Evol 12:e9305. https://doi.org/10.1002-ece3.9305

Cavieres L, Badano E, Sierra-Almeida A, Gómez-González S, Molina-Montenegro M (2006) Positive interactions between alpine plant species and the nurse cushion plant *Laretia acaulis* do not increase with elevation in the Andes of central Chile. New phytol 169:59-69. https://doi.org/10.1111/j.1469-8137.2005.01573.x

Costa J, Silva JS, Deus E, Pinho S, Pinto JF, Borralho N (2022) The genetics and ecology of post-fire *Eucalyptus globulus* recruitment in an isolated stand in Central Portugal. Forests 13:680. https://doi.org/10.3390/f13050680

da Silva PHM, Poggiani F, Sebbenn A, Mori E (2011) Can *Eucalyptus* invade native forest fragments close to commercial stands. For Eco Man 261:2075-2080. https://doi.org/10.1016/j.foreco.2011.03.001

dos Santos P, Matias H, Deus E, Águas A, Silva JS (2015) Fire effects on capsules and encapsulated seeds from *Eucalyptus globulus* in Portugal. Plant Ecol 216:1611-1621. https://doi.org/10.1007/s11258-015-0544-y

Faia J, Costa J, Araújo J, Borralho N, Marques C, Trindade H (2022) Impact of inbreeding on growth and development of young open-pollinated progeny of *Eucalyptus globulus*. iForest 15:356-362. https://doi.org/10.3832/ifor4012-015

Fernandes PM (2009) Combining forest structure data and fuel modelling to assess fire hazard in Portugal. Ann For Sci 66:415. https://doi.org/10.1051/forest/2009013

Fernandes P, Antunes C, Pinho P, Máguas C, Correia O (2016) Natural regeneration of *Pinus pinaster* and *Eucalyptus globulus* from plantation into adjacent natural habitats. For Eco Man 378:91-102. https://doi.org/10.1016/j.foreco.2016.07.027

Larcombe MJ, Silva JS, Vaillancourt RE, Potts BM (2013) Assessing the invasive potential of *Eucalyptus globulus* in Australia: Quantification of wildling establishment from plantations. Biol Invasions 15:2763-2781. https://doi.org/10.1007/s10530-013-0492-1

Lorentz KA, Minogue PJ (2015) Potential invasiveness for *Eucalyptus* species in Florida. Inv Plant Sci Manag 8(1):90-97. https://doi.org/10.1614/IPSM-D-14-00030.1

Padilla FM, Pugnaire FI (2006) The role of nurse plants in the restoration of degraded environments. Front Ecol Environ 4(4):196-202. https://doi.org/10.1890/1540-9295(2006)004[0196:TRONPI]2.0.CO;2

Rejmánek M, Richardson DM, Pyšek P (2005) Plant invasions and invasibility of plant communities. In: van der Maarel E, Franklin J (eds) Vegetation ecology. Blackwell Science, Oxford, UK, pp 332-355

Rezende GDSP, de Resende MDV, de Assis TF (2014) *Eucalyptus* breeding for clonal forestry. In: Fenning T (ed) Challenges and opportunities for the world's forests in the 21st century. Forestry Sciences 81. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7076-8_16

Silva JS, Vaz P, Moreira F, Catry F, Rego FC (2011) Wildfires as a major driver of landscape dynamics in three fire-prone areas of Portugal. Landsc Urban Plan 101(4):349-358. https://doi.org/10.1016/j.landurbplan.2011.03.001

Silva JS, Nereu M, Pinho S, Queirós L, Jesús C, Deus E (2021) Post-fire demography, growth and control of *Eucalyptus globulus* wildlings. Forests 12:156. https://doi.org/10.3390/f12020156

Stoneman GL (1994) Ecology and physiology of establishment of eucalypt seedlings from seed: A review. Aust For 57:11-29. https://doi.org/10.1080/00049158.1994.10676109