

Investigating the invasiveness of *Eucalyptus globulus* in Portugal: site-scale drivers, reproductive capacity and dispersal potential

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Abstract

Plantations of *Eucalyptus globulus* Labill. have expanded rapidly over the last four decades across temperate parts of the globe. There has been growing concern about the potential invasiveness of this species. Portugal grows more *E. globulus* than any other country (by area). This study investigates poorly understood factors that may influence wilding establishment and spread from Portuguese plantations. We assessed: site-scale factors influencing the presence and abundance of *E. globulus* wildlings along plantation edges, as well as the size and reproductive state of these wildlings; and the dispersal distances and developmental state of wildlings away from the plantations edge.

We found 1,630 *E. globulus* wildlings in 129 survey plots (10x10 m) established along the edge of 67 plantations. Half of the surveyed wildlings were taller than 1.3 m. The *presence* and *abundance* of *E. globulus* wildlings was mainly influenced by factors such as soil cover, the age of mother-trees and their reproductive output. Around 8% of the surveyed wildlings were reproductive, including wildlings less than 1.3 m tall. The proportion of reproductive wildlings was mainly influenced by wildling size, the location relative to the plantation edge and the annual number of frost days. In 49 survey transects outside plantations we found 641 *E. globulus* wildlings up to a distance of 76 m. Establishment distance was associated with natural drainage lines and the dominant wind direction. We discuss the relevance of the results in terms of the potential invasiveness of *E. globulus*.

Keywords

Blue gum; dispersal distance; eucalypt invasion; invasive alien species; natural establishment; naturalisation.

1. Introduction

Global species distributions are a consequence of diversification, dispersal and the rearrangement of land and sea over the geological time. Most taxa today are confined to geographic regions that are delineated by natural barriers to dispersal (Darwin 1859; Elton 1958). However, movement by humans has allowed many species to overcome such barriers, either through premeditated or inadvertent human will (Meyerson and Mooney 2007; Hulme 2009). Some of these exotic species have been a matter of great concern because of their ability to spread, causing negative ecological and economic impacts (Pimentel et al. 2001; Vilà et al. 2011).

Eucalyptus L'Hér. (Myrtaceae) is a large, diverse plant genus, comprising over 800 species, largely native to Australia (Nicolle 2018). Since the end of the 18th century several eucalypt species were introduced to different regions of the world (Potts 2004; Silva-Pando and Pino-Pérez 2016). Some eucalypt species were well adapted to the exotic environments and became widely exploited for timber, firewood, windbreaks, soil retention and, more recently, paper pulp production (Jacobs 1979; Potts 2004). Currently, it is estimated that eucalypt plantations cover around 20 million ha outside the native range (Rejmánek and Richardson 2011).

There has been a growing concern about the invasive potential of some eucalypt species. Several eucalypt species are considered to be naturalised (Pyšek et al. 2017) and a few species are regarded as invasive in different regions of the world (Rejmánek and Richardson 2013). Naturalised plant species have been defined as species able to generate reproductive offspring, normally near the parents, to form self-perpetuating populations, while invasive plant species are able to originate offspring distant from the parents, frequently causing negative ecological and economic impacts (Richardson et al. 2000; Blackburn et al. 2011).

A prior literature review, developed in the frame of another research initiative, revealed around 30 publications (papers and theses), assessing aspects of eucalypt invasiveness using wildling surveys or sowing experiments, mostly published in the last decade. These studies investigated

the invasiveness of dozens of eucalypt species in different countries, such as Brazil (e.g. Miolaro et al. 2017), South Africa (e.g. Forsyth et al. 2004), USA (e.g. Callaham Jr et al. 2013), Spain (e.g. Calviño-Cancela et al. 2018), Portugal (e.g. Fernandes et al. 2016) and Australia (e.g. Calviño-Cancela and van Etten 2018). *Eucalyptus globulus* Labill. is one of the most extensively cultivated eucalypt species (Rejmánek and Richardson 2011), and is probably the most widely studied eucalypt species in terms of invasiveness. Also, it is considered to be the most widely occurring invasive eucalypt species (in seven out of 15 geographic regions of the world; see Rejmánek and Richardson 2013).

Eucalyptus globulus is native to SE Australia, including the Bass Strait Islands and Tasmania (Jordan et al. 1994). It was introduced throughout the world in the 19th century and plantations expanded rapidly in several countries in the late 20th century, especially to feed the paper and pulp markets (Potts et al. 2004). Portugal grows more *E. globulus* than any other country (by area), covering more than 800,000 ha, around 9% of the country (ICNF 2013; Deus et al. 2018a). The species has a wide environmental tolerance and is able to naturally establish in different regions of Portugal (Catry et al. 2015).

Studies assessing factors associated with *E. globulus* wildling establishment have shown that climate (Larcombe et al. 2013; Catry et al. 2015; Fernandes et al. 2017; Fernandes et al. 2018), plantation-related variables such as plantation size and age (Águas et al. 2017; Fernandes et al. 2018), fire (Larcombe et al. 2013; Calviño-Cancela et al. 2018), post-fire management (Águas et al. 2014), soil cover and disturbances (Kirkpatrick 1977; Fernandes et al. 2018), and the invaded land cover type (Calviño-Cancela and Rubido-Bará 2013; Fernandes et al. 2016; Fernandes et al. 2018) can all be important. Despite previous work investigating *E. globulus* invasiveness, knowledge gaps persist.

Little is known about the demographics of *E. globulus* wildling populations. Understanding the developmental stage of wildlings (e.g. size and transition to adult foliage) provides information about demography such as the number of cohorts and their survival rates, and is also crucial for

making conclusions about effective recruitment because small *E. globulus* wildlings experience high levels of mortality (Calviño-Cancela and Rubido-Bará 2013; Fernandes et al. 2017). Few studies assess the height of naturally established wildlings (e.g. Águas et al. 2014; Fernandes et al. 2018) or the transition to adult foliage (e.g. Calviño-Cancela and van Etten 2018). Moreover, to our knowledge, no study investigated the occurrence of wildlings bearing reproductive organs. The onset of sexual maturity is of particular importance, because it is key for determining *naturalisation* and *invasion* (Richardson et al. 2000), and has not been studied in exotic *E. globulus*.

Few studies investigated the natural establishment of *E. globulus* wildlings along the edges of plantations. The edges of plantations are of particular importance because they represent the point of potential escape for wildlings from cultivation, and observations on *E. globulus* plantations in Australia show that flowering seems to be more abundant in the trees located along the plantation edges (Barbour et al. 2008), meaning that propagule pressure is probably higher at these locations. Moreover, forest edges form transition zones between two adjacent ecosystems, featuring particular biotic (e.g. species diversity) and abiotic (e.g. microclimate) conditions (Murcia 1995). However, among the few studies investigating wildling establishment along plantation edges, the role of site-scale factors such as soil cover, local topography or slope aspect, was either not investigated (Águas et al. 2017) or inconclusive (Larcombe et al. 2013). It is known that fine-scale factors such as soil moisture, vegetation cover and slope are important in native eucalypt recruitment in Australia (Bailey et al. 2012; Wilson and Gibbons 2014). A recent study conducted in Portugal, using survey plots outside *E. globulus* plantations, also emphasised the importance of site-scale factors such as soil cover and soil disturbance in *E. globulus* recruitment (Fernandes et al. 2018).

Seeds of *E. globulus* do not have any long-distance dispersal adaptation, being normally dispersed by gravity and wind (Cremer 1977; Booth 2017). Nevertheless, seeds of *E. globulus* have been recorded 80 m away from plantations in NW Spain (Calviño-Cancela and Rubido-Bará 2013). Previous studies assessing the dispersal of wildlings from plantations (Calviño-Cancela and

Rubido-Bará 2013; Larcombe et al. 2013; Fernandes et al. 2016) have not considered the importance of the primary vectors of dispersal, wind and water. Wind is probably the primary dispersal vector of eucalypt seeds (Cremer 1977; Calviño-Cancela and Rubido-Bará 2013), and the orientation of the plantation in relation to the prevailing wind may be an important factor determining the dispersal distances (McBride et al. 1988). Water has also been implicated in the dispersal of eucalypt seeds (Kirkpatrick 1977; Forsyth et al. 2004), and Larcombe et al. (2013) observed *E. globulus* wildlings established 175m from a plantation down slope in a drainage line. Therefore, the presence of drainage lines (and presumably ephemeral running water) may be associated with increased wildling dispersal distances.

The goal of this study is to contribute to the understanding of *E. globulus* invasiveness, by addressing some important, but poorly explored topics. We surveyed wildling establishment along the edges of *E. globulus* plantations, and assessed dispersal into adjacent areas in Central Portugal. In particular, this study aimed to: a) assess the local site-scale factors influencing presence and abundance of *E. globulus* wildlings; b) quantify the size, foliage development (adult/juvenile), and proportion of reproductive wildlings; c) investigate the effective dispersal distance of wildlings from plantations, including the distribution of developmental classes; and d) assess factors affecting dispersal distance.

2. Material and Methods

2.1 Study region

The study was conducted in Central Portugal (Fig. 1). Climate is typically Mediterranean, with hot, dry summers and moist winters. Mean annual precipitation is 652 mm, registering minimum values in July (4 mm) and maximum values in November (107 mm). Mean annual temperature is 17 °C, ranging from 10 °C in January to 24 °C in August (climatic data retrieved from IPMA 2018). The topography is irregular, featuring predominantly rugged terrain of hills and valleys, and river plains to a lesser extent. Sampling units, composed of survey plots and survey transects associated to *E. globulus* plantations, were located within latitudes 39°32'02" N and 38°47'04" N (distributed within a latitudinal distance of 83 km), and longitudes 7°46'52" W and 9°00'08" W (longitudinal distance of 106 km). Altitude of the sampling units ranged between 30 and 307 m. Plantations of *E. globulus* are widespread in this region, occupying c. 11% (c. 92,000 ha) of the area delimited by the referred coordinates.

2.2 Field surveys

2.2.1 Survey along plantation edges

We performed a survey along the edges of *E. globulus* plantations aiming to assess wildling development and the influence of site-scale factors on the establishment of *E. globulus* wildlings. All *E. globulus* plantations were owned by a Portuguese pulp company (Altri Florestal S.A.). The age of plantations ranged between 4 and 42 years (mean=23.4; SD=8.1). Since *E. globulus* reaches sexual maturity in plantations at 3-4 years of age (Potts and Gore 1995; Potts et al. 2008), plantations were composed of potentially reproductive individuals. The maximum stem age of the surveyed plantation was 12 years-old, meaning that flowering could have started 8-9 years before. In Portugal, plantations are typically managed by coppicing every 10-12 years for 2-3 rotations.

The survey design was similar to a study performed in Australia by Larcombe et al. (2013). The edge (perimeter) of a plantation was defined as a virtual line crossing the boles of the outer trees.

The perimeter of the plantations was divided in different edges according to the edge aspect. In this way, a single plantation could comprise distinct edges, which were assessed separately using different transects. In each transect, the survey was performed on 10x10 m plots disposed along the plantation edge. In this way, the perimeter of the plantation divided the plot into two halves, i.e. two adjacent 5x10 m subplots, one inside (*inside subplot*) and one outside (*outside subplot*) the plantation. We aimed to accomplish a paired-plot survey within each transect, featuring a plot where wildlings were present (*presence plot*) combined with a plot where wildlings were absent (*absence plot*).

A *presence plot* was established in the first opportunity to frame a minimum of five wildlings within the plot area, regardless of their size and location (*inside* and/or *outside subplots*). The corresponding *absence plot* was established in the first available wildling-free plot area in either direction along the plantation edge, at a minimum distance of 50 m from the *presence plot* and 15 m away from any other wildling. Minimum distance between *presence plots* was 200 m. Due to the recurrent presence of *E. globulus* wildlings, it was not possible to couple an *absence plot* to every *presence plot*. Therefore, the survey included more *presence* than *absence plots*. We discarded all plantation edges distanced less than 50 m from another *E. globulus* plantation to reduce the chances of registering wildlings originating from other plantations. In the *outside subplot*, we subtracted the area of any surface unsuitable for wildling establishment (e.g. road).

In the *presence plots*, for each subplot (*inside* and *outside*), *E. globulus* wildlings were counted and categorised according to four size classes: ≤ 0.5 m (*size 1*); 0.5-1.3 m (*size 2*); 1.3-3.0 m (*size 3*); > 3 m (*size 4*). For each size class, we also registered the number of *reproductive wildlings*, i.e. wildlings bearing reproductive organs such as flowers or fruits. Wildlings were distinguished from planted individuals by their irregular location and size, within the regularly spaced and similarly sized planted *E. globulus* trees. Wildlings were also distinguished from resprouts by the presence of a lignotuber. *Eucalyptus globulus* is a proficient resprouter, able to regenerate through epicormic strands and from basal buds (Catry et al. 2013).

In each survey plot, including *presence* and *absence plots*, we collected the following data: a) *number of capsules* on each of the ten planted eucalypts closest to the plot centre (visual estimate

using binoculars), within a maximum distance of 25 m (categorised as 0: no capsules; 1: between 1-10 capsules; 2: 11-100 capsules; 3: 101-1000 capsules; 4: >1000 capsules); b) *edge aspect*, i.e. direction of exposure of the plantation edge (four cardinal directions); c) *plot topography*, i.e. topographic format of the plot surface (flat; valley; slope; ridge); d) *plot aspect*, direction of terrain inclination (flat; four cardinal directions, later reclassified north and west to *wet*, and south and east to *dry*); e) *plot slope* (degrees of inclination); and f) the percentage of the plot covered by four soil cover types: bare ground, litter, herbs and shrubs.

A total of 103 edge transects were performed in 67 *E. globulus* plantations, including 129 *presence plots* and 53 *absence plots*. Field work was conducted between January and May 2014.

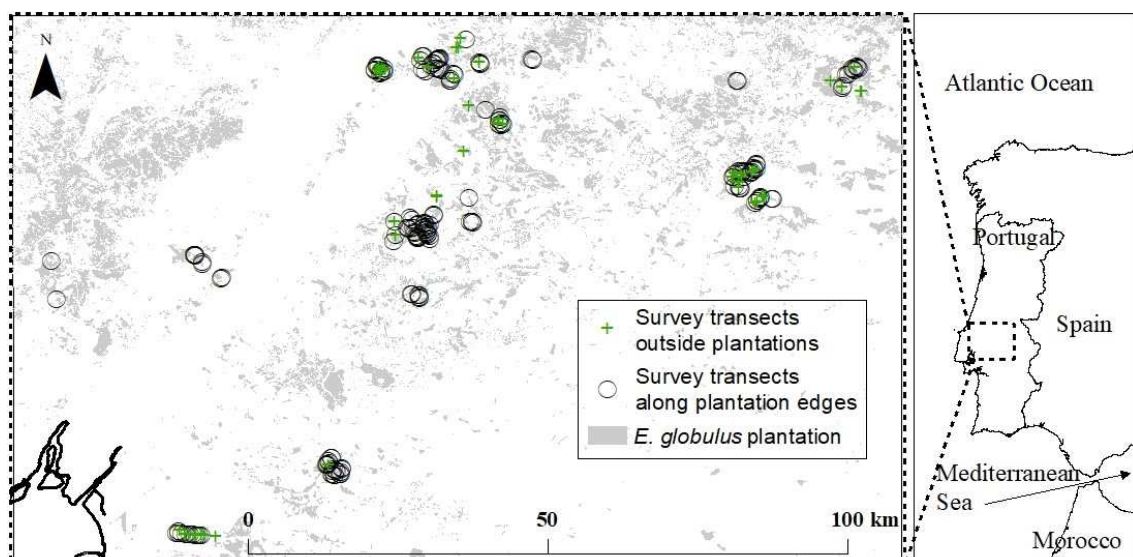


Fig. 1 - Distribution of *Eucalyptus globulus* plantations and sampling areas in the study region

2.2.2 Survey outside plantations

We surveyed *E. globulus* wildlings established outside plantations using transects perpendicular to the plantation edges. This survey aimed to record the distance of wildling dispersal from the plantation together with wildling development. The establishment of a perpendicular transect was triggered by the detection of one *E. globulus* wildling at a distance greater than 10 m from the plantation edge. Transects had a maximum length of 80 m, corresponding to the maximum seed

dispersal distance registered in *E. globulus* plantations in NW Spain (Calviño-Cancela and Rubido-Bará 2013). Minimum distance between transects was 100 m to avoid registering the same wildlings twice. Transects were established in areas with no other *E. globulus* plantations nearby, to avoid recording wildlings from other plantations.

The survey was conducted by one observer walking along the transect. Each step, previously calibrated, corresponded to 1 m length. At each step, the researcher looked at both sides and recorded the number and developmental stage of wildlings at that specific distance. Since the observer should not move away from the transect in order to have the same detection criteria across transects, we decided not to collect any specific traits which would require closer look, such as plant height and the existence of reproductive organs. In alternative, we characterised the developmental stage of wildlings using the conspicuous juvenile/adult foliage of *E. globulus*. *Eucalyptus globulus* is strongly heteroblastic, featuring strikingly different juvenile and adult foliage, making this developmental transition conspicuous and easily detectable (Johnson 1926; Velikova et al. 2008). We used the conservative assumption that wildlings with juvenile foliage are non-reproductive because the onset of sexual reproduction does not normally occur until after the transition to adult leaves (Potts et al. 2008), although juvenile wildlings with reproductive structures were identified in this study (see results; Fig. 2). For each transect, we collected the following data: *transect land cover*, i.e. predominant land cover along the transect (agriculture; shrubland; pine plantation; riparian zone; *montado*; paddock); *drainage line presence*, i.e. occurrence of a natural drainage line running away from the plantation edge (yes; no). *Montado* is a traditional agroforestry system in Portugal characterised by an open forest of native oaks. A total of 49 survey transects were performed outside 33 *E. globulus* plantations (Fig. 1). Field work was conducted between January and May 2014.

2.3 Data analysis

2.3.1 Wildling presence

This analysis aimed to investigate the site-scale factors (see Section 2.2.1) influencing the *presence* of *E. globulus* wildlings, regardless of their size, along the edge of plantations. We used wildling presence-

absence in the 10x10 m survey plots as a binary response variable. This analysis contrasts local conditions that are very adequate (at least five plants in 100 m²) for wildling establishment (recruitment and persistence in *presence* plots) with local conditions that are inadequate for establishment (*absence* plots). *Presence* was preferred to *density* as a dependent variable, because of poor performance of the *density* models that were produced at an exploratory stage of the analysis. Explanatory variables included the site-scale variables collected in each subplot (see Section 2.2.1) and the *stem age* of the planted *E. globulus* trees, i.e. the time (years) since planting or coppicing, information that was provided by the plantation owner. The variable *stem age* was included as a covariate to remove predictable variation on some site-scale factors associated with tree development. For instance, older stems should correspond to lower soil exposure and higher reproductive potential. Also, this factor (*stem age*) is constant among survey plots located within the same plantation. The reproductive capacity of the plantation was determined using the *maximum capsule score* from the 10 trees associated with the plot. We used logistic regression to assess the influence of site-scale factors on the presence of *E. globulus* wildlings. Due to the hierarchical nature of our sampling design (*transects* nested within *plantations*), we used generalised linear mixed models (GLMM) with a binomial distribution and logit link function, using the R package *lme4* (Bates et al. 2017). We fitted a model using *presence-absence* as the dependent variable, with *transects* nested within *plantation* as random effects. In order to avoid multi-collinearity between explanatory variables we computed variance inflation factors (VIF) to select the final set of potential predictors with $VIF < 4$ (Zuur et al. 2009) using the R package *usdm* (Naimi 2017).

The following fixed effects were included in this order: *stem age*, *bare ground cover*, *litter cover*, *shrub cover*, *herb cover*, *plot topography*, *plot slope*, *maximum capsule score*, *plot aspect (wet and dry)* and *edge aspect*. We used a backwards step model simplification process where non-significant terms were removed from the model, starting with categorical terms with the highest *P*-value. The reduced model was compared to the previous model using the likelihood ratio test. If the reduced model was significantly worse than the previous model, the dropped term was reinstated. Alternatively, the reduced model was accepted. This process was repeated until no non-significant terms remained. Residual diagnostics were conducted on simulated scaled residual plots, produced using the R package *DHARMA* (Hartig 2017). This procedure produces interpretable residuals from hierarchical mixed models. Model

fit was assessed by conditional R^2 (Nakagawa and Schielzeth 2013), using the R package *piecewiseSEM* (Lefcheck 2016).

2.3.2 Wildling abundance

We used a paired Wilcoxon test to compare the density (number of wildlings per area) of each wildling size class between the *inside* and *outside subplots*, retaining exclusively the *presence plots* (plots featuring a minimum of five wildlings).

We explored the influence of site-scale factors on the abundance of young plants, as an indicator of site suitability for recruitment. As a response variable, we used wildling density (number of wildlings per area). We restricted this analysis to the abundance of smaller wildlings (*size 1*; <50 cm) in the *inside subplots* of the *presence plots*. This criterion was used for two reasons: first, younger wildlings should reflect more accurately the site-scale conditions registered at the date of the survey; second, *inside subplots* are less prone to disturbances (e.g. vehicles; people). Unlike the previous analysis, we discarded all *absence plots* in order to retain only locations where wildlings find conditions to establish. We used the same set of explanatory variables as in the presence-absence analysis, including *stem age* and site-scale variables (see Section 2.2.1).

Since density data were highly skewed and could not be transformed to meet the assumptions of generalised models, we used regression trees as an alternative approach. We chose multivariate regression trees (MRT) to assess wildling density. Among other features, MRT provide a very clear picture of the structure of the data and provide a highly intuitive insight into the kinds of interactions between variables (Crawley 2012). MRT result from the successive splitting of the sites (survey plots) into two groups based on a threshold from one of the explanatory variables, so that each group presents a minimum dissimilarity (De'ath 2002). The minimum number of observations to split (starting with $n=129$) was set to 20 and the minimum number of observations in a terminal node was set to 6. The MRT was built using the R packages *rpart* (Therneau et al. 2018) and *rpart.plot* (Milborrow 2017).

2.3.3 Occurrence of reproductive wildlings

This analysis aimed to understand the factors influencing the occurrence of reproductive wildlings. We used the proportion of reproductive wildlings in each plot as the response variable. Smaller wildlings (size 1 and 2; <1.3 m) were excluded from this analysis since only a very small percentage was found to be reproductive (<0.5% in both size classes). Therefore, our sampling units were the *inside* and *outside subplots* featuring size 3 (1.3-3 m) and/or size 4 (>3 m) wildlings. We used a larger set of explanatory variables when compared to the previous analyses because the occurrence of reproductive eucalypt wildlings has been poorly investigated. Explanatory variables included: a) the set of site-scale variables used in previous analyses (see Section 2.2.1); b) the subplot location (*inside* or *outside subplots*); c) the number of wildlings of *size 3* and *size 4* (two variables); d) plantation-related variables, provided by Altri Florestal S.A., including the *plantation age* (time since planting), *plantation rotation* (first; second; third), the *stem age* (time since planting or coppicing) and *site index* (height of the dominant trees at ten years old); e) climate, altitude and soil variables, retrieved from APA (2018), namely: the *mean annual temperature* (°C; average 1931-60), the *mean annual precipitation* (mm; average 1931-60), the *mean annual number of frost days* (average 1941-60), *altitude* (meters) and the *soil type* (cambisol; lithosol; luvisol).

In order to assess the factors influencing the proportion of reproductive wildlings, we used a generalised linear model (GLM) with a binomial distribution and logit link function. We computed VIF (>4) to check for collinearity between variables. Model simplification and validation followed the procedure previously described for the *presence* model (Section 2.3.1). Model fit was assessed using the proportion of explained deviance.

2.3.4 Distance of establishment from plantations

This analysis aimed to investigate the factors influencing the distance of establishment of *E. globulus* wildlings outside plantations. For this analysis, we used linear mixed models (LMM), with the *nlme* R package (Pinheiro et al. 2018). The dependent variable was the distance of wildling establishment from plantations. Since the dispersal distance of eucalypt wildlings has been less well investigated in eucalypt

ecology, we included the same wider set of explanatory variables as in the analysis of reproductive wildlings (see Section 2.3.3), without the site-scale variables. Additionally, we included the variable *drainage line presence* (see Section 2.2.2) and variables retrieved from a digital terrain map: a) *transect aspect*, i.e. direction of exposure of that area (flat; four cardinal directions); b) *transect slope*, i.e. terrain inclination in that area (degrees). *Land cover* was not included in the analysis because some land cover types were underrepresented. Topographical variables (aspect; slope) were provided by Altri Florestal S.A. and originally retrieved from 1:25,000 cartography produced by the Portuguese Army Geographical Institute (IGeoE). Most transects were performed in downslope areas and *transect aspect* coincided roughly with the cardinal direction of the transect. The *transect ID* was included in the model as a random effect. We used VIF (>4) to discard correlated variables. Model simplification and validation followed the previously described procedure. As in the *presence* model (Section 2.3.1), model fit was assessed using the conditional R^2 . All statistical analyses were performed using R software (R Core Team 2017).

3. Results

3.1 Wildling establishment along plantation edges

A total of 1,630 *E. globulus* wildlings were recorded in the 129 *presence plots*. Wildlings of *size 2* (0.5-1.3 m) were the most frequent (n=575; 35.3% of total), followed by *size 3* (1.3-3 m; n=481; 29.5%), *size 4* (>3 m; n=334; 20.5%) and *size 1* (< 0.5 m; n=240; 14.7%). The overall wildling density (all plants in total area of *presence plots*) was 1461.2 wildlings ha⁻¹, being higher in the *inside subplots* (1771.7 wildlings ha⁻¹) compared to the *outside subplots* (1044 wildlings ha⁻¹). Taking into account the factor wildling size, the overall wildling density was higher in the *inside subplots* for wildlings of *size 2* (650.5 vs. 334.0 wildlings ha⁻¹), *size 3* (556.7 vs. 262.6 wildlings ha⁻¹) and *size 4* (405.0 vs. 157.6 wildlings ha⁻¹), while it was lower for *size 1* wildlings (159.5 vs. 289.9 wildlings ha⁻¹), compared to the *outside subplots* (Fig. 3). The maximum wildling density was 6,000 wildlings ha⁻¹, registered in a 5x10 m *inside subplot*, composed exclusively of *size 1* (<0.5 m) wildlings. A paired Wilcoxon test showed that differences in wildling density between the *inside* and *outside subplots* were significant for wildlings of

size 2 ($p=0.03$), size 3 ($p<0.001$) and size 4 ($p<0.001$). We assessed the reproductive capacity of 1,820 neighbour *E. globulus* trees in the survey plots, of which 69% possessed capsules.



Fig. 2 - Reproductive *Eucalyptus globulus* wildlings of size 1 (<0.5 m) and size 2 (0.5-1.3 m). Red arrows indicate flowers

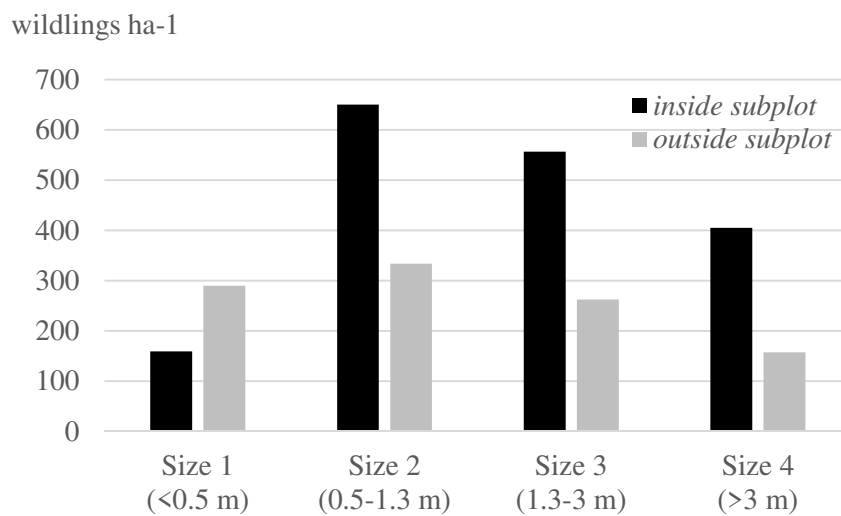


Fig. 3 – Overall density (all sampled plants / total surveyed area) of *Eucalyptus globulus* wildlings in the presence plots according to wildling size classes and subplot type

Among the 1,630 *E. globulus* wildlings found, there were 124 (7.6%) reproductive wildlings, i.e. sexually mature wildlings, possessing flowers or capsules. The overall density of reproductive wildlings was 115.6 wildlings ha⁻¹, being higher in the inside subplots (146.9 wildlings ha⁻¹) compared to the outside subplots (63.0 wildlings ha⁻¹). We found one reproductive size 1 wildling (0.4% of the total size

1 wildlings) and two reproductive *size 2* wildlings (0.3%; see Fig. 2). The proportion of reproductive wildlings was higher in *size 3* (5%; n=24) and *size 4* wildlings (29%; n=97).

3.1.1 Factors influencing wildling presence

The GLMM, performed to assess the influence of site-scale factors on wildling presence (Table 1), explained 32% of the variance. The variable *stem age*, included as a covariate, was retained in the model, but it was not significant. Three variables showed a significant, positive influence on wildling presence: *litter cover*, *plot slope* and *maximum capsule score*. The variable *edge aspect* also had a significant influence, particularly a negative effect of the *west* aspect on wildling presence.

Table 1 - Results from a binomial generalised linear mixed models (GLMM) produced to assess the influence of site-scale factors on the presence and absence of *Eucalyptus globulus* wildlings at the edges of plantations. The reference level of the categorical variable is shown in brackets

Variable	Coefficient	Std. Error	Z value	P-value
Intercept	-0.899	0.815	-1.102	ns
Stem age	-0.137	0.091	-1.498	ns
Litter cover	0.036	0.009	3.992	***
Plot slope	0.096	0.033	2.842	**
Max. caps. score	0.383	0.169	2.262	*
Edge aspect				
(<i>East</i>)				
<i>North</i>	-0.934	0.534	-1.749	ns
<i>South</i>	0.136	0.527	0.259	ns
<i>West</i>	-1.377	0.534	-2.579	**

Significance P-value: $\leq 0.001 = ***$; $\leq 0.01 = **$; $\leq 0.05 = *$; $> 0.05 = ns$

3.1.2 Factors influencing wildling abundance

The MRT, used to assess the influence of site-scale factors on the abundance of *size 1* (<0.5 m) wildlings in the *inside subplots* (see Section 2.3.2), created six clusters of plots. Four variables, mostly soil cover-

related, were responsible for the five partitions originating the six clusters of plots (Fig. 4). Wildling density was found to be negatively related with *shrub cover* (first two partitions). The remaining plots were split by *stem age* of 10 years (third partition), where plots under trees with older *stem age* registered higher wildling density. The percentage of *bare ground* created the fourth partition, where plots featuring higher proportion of *bare ground* had higher wildling density. The fifth partition was created by the percentage of *herb cover*, being negatively related to wildling density. The whole tree explained 22 % of the variance.

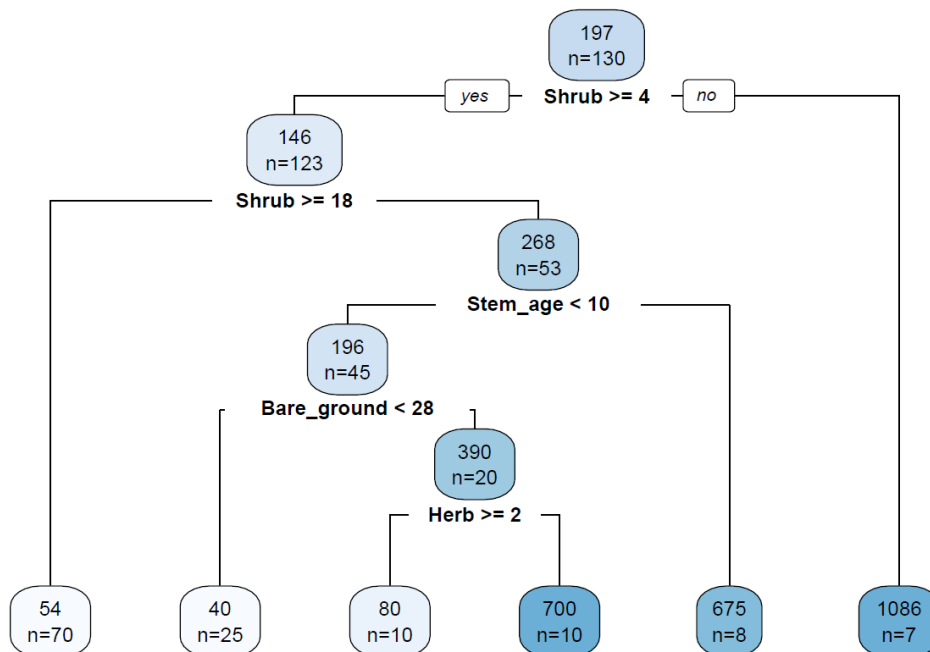


Fig. 4 - Multivariate Regression Tree (MRT) of *Eucalyptus globulus* wildling density (plants ha⁻¹; only wildlings up to 0.5 m tall – *size class 1*) using site-scale variables and *stem age* as explanatory variables (see Section 2.3.2). Each splitting node is represented by a box featuring the average wildling density (number above) and the corresponding number of plots (number below). Colour darkness in each box is proportional to the fitted value. The splitting variable and the threshold value responsible for the partition is presented below the box. The length of each branch is proportional to the difference in deviance between each node and the sums of the nodes below

3.1.3 Factors influencing reproductive wildlings

The final GLM produced to explore the factors influencing the proportion of reproductive *E. globulus* wildlings along the edge of plantations, retained 11 variables, but only seven were significant ($p < 0.05$). The proportion of reproductive wildlings was positively influenced by the location in the *outside subplots*, a higher *site index*, *wet* aspects, high abundance of *size 4* ($> 3\text{m}$) wildlings and older *stem age* in the cultivated trees. On the contrary, higher *altitude* and increased *number of frost days* negatively affected the proportion of reproductive wildlings (Table 2). The model explained 53% of deviance.

Table 2 - Results of a generalised linear model (GLM) used to assess the factors influencing the proportion of reproductive *Eucalyptus globulus* wildlings along the edges of plantations. The reference level of categorical variables is shown in brackets

Variable	Coefficient	Std. Error	Z value	P-value
Intercept	11.380	2.233	-5.094	***
Subplot location				
<i>(Inside subplot)</i>				
<i>Outside subplot</i>	0.824	0.255	3.222	**
Bare ground	0.016	0.009	1.801	ns
Shrub cover	0.012	0.006	1.880	ns
Site index	0.232	0.085	2.727	**
Soil type				
<i>(Cambisols)</i>				
<i>Lithosols</i>	-15.590	911.900	-0.017	ns
<i>Podzols</i>	0.571	0.333	1.715	ns
Altitude	-0.008	0.002	-3.822	***
Plot aspect				
<i>(Dry)</i>				
<i>Wet</i>	0.577	0.265	2.176	*
Plot topography				
<i>(Flat)</i>				
<i>Ridge</i>	-17.470	1,859.000	-0.009	ns
<i>Slope</i>	0.032	0.279	0.115	ns
<i>Valley</i>	-0.504	0.546	-0.922	ns
Wildling size				
<i>(Size 3; 1.3-3 m)</i>				
<i>Size 4; >3 m</i>	0.152	0.024	6.277	***
Stem age	0.600	0.113	5.279	***
Annual number frost days	-0.272	0.081	-3.343	***

Significance P-value: $\leq 0.001 = \text{***}$; $\leq 0.01 = \text{**}$; $\leq 0.05 = \text{*}$; $> 0.05 = \text{ns}$

3.2 Wildling establishment outside plantations

Most survey transects were established in *montado* (n=27; 55%) and shrubland (n=14; 29%). A few transects were established in paddock areas (n=5; 10%), and one transect (2%) was established in a pine stand, another in an agriculture area and another in a riparian area. We found a total of 641 *E. globulus* wildlings along the perpendicular transects, mostly established in *montado* (53.2%) and shrubland (39.9%). On average, we found 13.1 wildlings per transect (SD=14.6; min.=1; max.=70).

Around 75% of the *E. globulus* wildlings escaped from plantations were found within a distance of 15 m from the plantation edge, and c. 91% within 30 m. There were 18 wildlings (c. 3%) located beyond 50 m from the edge of plantations. The maximum distance recorded was 76 m. Around 59% of the *E. globulus* wildlings recorded outside plantations had adult foliage. The proportion of wildlings exhibiting adult foliage increased with the distance from the plantations (Fig. 5).

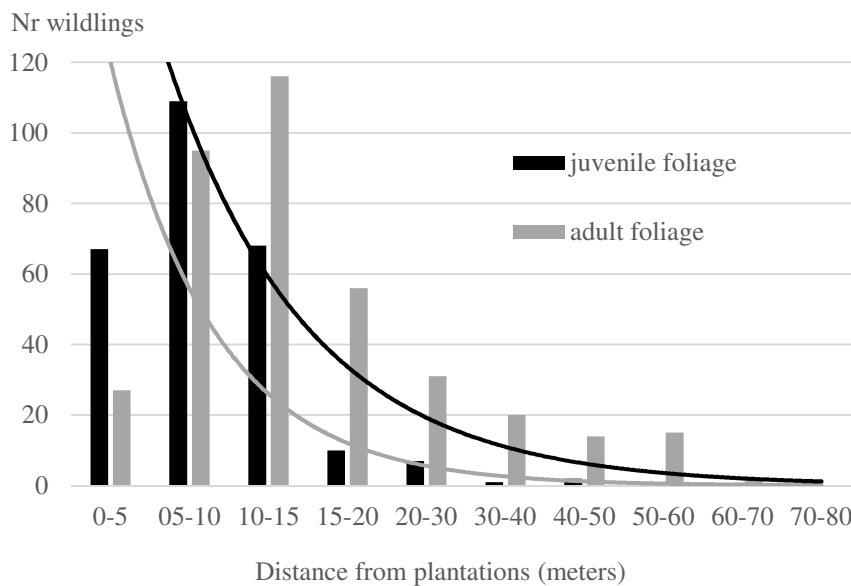


Fig. 5 - Number of *Eucalyptus globulus* wildlings with juvenile and adult foliage according to distance from the plantation. The general decreasing trend is illustrated using fitted negative exponential curves

3.2.1 Factors influencing the distance of establishment

The LMM, computed to understand the factors influencing the dispersal distance of *E. globulus* from plantations, highlighted the importance of *drainage line presence*, the *soil type* and the *transect aspect* (Table 3). The presence of natural drainage lines along the surveyed area enhanced the distance of wildling establishment. On the other hand, the distance of establishment was negatively influenced by west exposures and, with less importance, by the occurrence of podzols. The fitted model with these three variables explained 18% (Nagelkerke R^2) of the observed variance.

Table 3 - Results from a linear mixed model (LMM) showing the variables influencing the dispersal distance of *Eucalyptus globulus* from plantations into adjacent areas. The reference level of categorical variables is shown in brackets

Variable	Coefficients	Std. Error	<i>t</i>	<i>P</i> -value
Intercept	20.694	3.450	5.600	***
Drainage line (Absence)				
<i>Presence</i>	18.350	1.910	9.609	***
Transect aspect (East)				
<i>West</i>	-9.9202	3.392	2.924	**
<i>North</i>	-5.281	3.586	-1.473	ns
<i>Flat</i>	-3.160	3.062	-1.032	ns
<i>South</i>	3.398	3.542	0.959	ns
Soil type (Cambisols)				
<i>Podzols</i>	-7.177	3.127	-2.295	*
<i>Lithosols</i>	4.070	4.798	0.848	ns

Significance *P*-value: $\leq 0.001 = ***$; $\leq 0.01 = **$; $\leq 0.05 = *$; $> 0.05 = ns$

4. Discussion

4.1 Wildling establishment along plantation edges

Most previous studies investigating the natural establishment of exotic eucalypts do not consider wildling development, especially the onset of sexual reproduction. This study provides evidence of the effective recruitment of *E. globulus* wildlings along the edges of plantations. Others have reported a high rate of mortality of *E. globulus* wildlings in the first months after germination (Fernandes et al. 2017). Calviño-Cancela and Rubido-Bará (2013) found that the survival probability of *E. globulus* seedlings increases every month, reaching 82% between the 6th and 8th months. We found no previous information regarding the growth rate of naturally established *E. globulus* wildlings. However, based on recent field observations in Portugal, we are confident that wildlings of *size 3* (1.3-3 m) and *size 4* (>3 m), which comprise half of the surveyed wildlings in this study, should be more than six months old, and thus have high chances of survival.

We recorded higher wildling abundance in the *inside subplots*. A similar trend was found in other studies (Larcombe et al. 2013; Fernandes et al. 2016; Águas et al. 2017). This discrepancy between *inside* and *outside subplots* is probably related to the higher frequency of disturbances outside plantations, such as the passage of vehicles and people, or fuel reduction operations for fire prevention, targeting especially larger plants, as hypothesised by other studies (Larcombe et al. 2013; Águas et al. 2017). This discrepancy between *inside* and *outside subplots* is more significant for older plants (>50 cm; sizes 2, 3 and 4). This is consistent with the findings of a similarly study in Australia by Larcombe et al. (2013), and seems to support the idea that disturbances such as multi-year management cycles or stochastic events outside the plantation may periodically reduce abundance.

4.2 The role of site-scale factors on wildling establishment

The *presence* of *E. globulus* wildlings along the edge of plantations was affected by factors as diverse as soil cover, topography, propagule pressure and aspect. We note that because *presence* considered wildlings of all sizes, some more dynamic site-scale variables, such as *soil cover* and *maximum capsule score*, may not portray the conditions at the time of wildling establishment.

Among the soil cover variables, *litter* positively affected wildling presence, being the most significant variable. We did not discriminate the type of litter but it included mostly eucalypt leaves, branches and bark, even though litter from other plant species was also common and, in a few locations, quite conspicuous. The amount of litter may reflect indirectly important factors for wildling establishment. For instance, litter is positively related to the residence time of the plantations, which in its turn indicates a greater propagule pressure and thus more chances of wildling establishment. More litter also suggests that the plantation had not been recently managed, therefore favouring wildling persistence. Likewise, more litter should correspond to lower cover of other categories, in particular shrubs or herbs which compete for light and soil resources, and thus may limit the establishment of *E. globulus* wildlings, which are particularly sensitive to competition (Kirkpatrick 1977). In fact, it is known that *E. globulus* litter causes negative allelopathic effects on other plant species (Souto et al. 1994; Souto et al. 2001).

Plot slope was also found to affect positively the presence of *E. globulus* wildlings. Catry et al. (2015) found a similar result in roadsides and hypothesised that, in steeper areas, plant competition is lower due to greater soil erosion, and there is lower likelihood of waterlogging. In our case, it is also likely that areas featuring steeper slopes are managed less frequently or less effectively in terms of vegetation removal, due to technical and operational limitations.

The *maximum capsule score* (an indicator of reproductive output) was a significant factor positively affecting the presence of *E. globulus* wildlings, which is consistent with the findings of a similar study in Australia by Larcombe et al. (2013). It is known that propagule pressure has a central importance in plant invasions (Simberloff 2009). In our study, locations where trees produce more flowers and seeds are also likely to have wildlings.

The *west exposure* of the edge of the plantation, was the only site-scale factor influencing negatively the presence of *E. globulus* wildlings. Winds in this region are predominantly from northwest, meaning that west edges are more frequently exposed to wind. Therefore, one explanation may be related with higher seed-shadow drift towards the inner part of the stand, resulting in lower wildling density close to the edge of the plantation. Another possible explanation may be that western plots have no shelter against predominant winds, which may act together with higher sunlight exposure and higher temperatures,

resulting in higher drieress and therefore worst conditions for wildling establishment (Stoneman 1994; Wang et al. 1998).

The analysis of wildling abundance, using Multivariate Regression Trees (MRT), aimed to detect direct correspondence between local conditions and the abundance of young plants (<0.5 m; see Section 2.3.2). We found that *shrub cover*, *bare ground cover* and *herb cover*, were responsible for four of the five data partitions in the MRT. These results seem to highlight the importance of competition with other vegetation in the emergence and development of *E. globulus* wildlings, because shrubs and herbs negatively affected wildling density, while bare ground had the opposite effect. The findings are also in line with the known preference of eucalypt wildlings for disturbed, competition-free areas (Kirkpatrick 1977; Fernandes et al. 2018). The positive effect of *stem age* on wildling abundance should reflect the positive influence of residence time and the increasing propagule pressure originating from the cultivated trees as they grow older (Richardson 1998; Lockwood et al. 2005; Simberloff 2009).

4.3 Reproductive offspring

To our knowledge, this is the first study to record and assess the reproductive status of *E. globulus* wildlings. Reproductive *E. globulus* wildlings were not found in a similar survey conducted on *E. globulus* plantations in Australia (Larcombe et al. 2013). Importantly, flowering was identified in juvenile wildlings (Fig. 2), which does not normally occur until after the transition to adult leaves (Potts and Gore 1995). Early flowering is often a target of breeding programs (Flachowsky et al. 2011), and may have been specifically targeted to accelerate production during the development of the *E. globulus* industry in Portugal. Time to first flowering is under strong genetic control in *E. globulus* (Jordan et al. 1999) and is highly heritable (Chambers et al. 1997). A short juvenile period was also found for *E. grandis* in South Africa (Musengi and Archibald 2017) and is a common trait among some of the most invasive plant species (Rejmánek and Richardson 1996; Grotkopp et al. 2002).

Among the factors influencing the proportion of reproductive wildlings, wildling size (height) was the most significant. Larger wildlings (*size class 4*; >3 m) were more likely to be reproductive, which is similar to observations in South Africa for *E. grandis* wildlings (Musengi and Archibald 2017). *Stem age* was the second most influential factor, also showing a positive influence. Again, older plantations

have more chance of accommodating older (larger) wildlings, which in its turn have a greater likelihood of being reproductive.

The *number of frost days* negatively affected the proportion of reproductive wildlings. Countrywide surveys in Portugal suggest that higher numbers of frost days limits wildling abundance (Catry et al. 2015; Deus et al. 2016). This has been associated with the sensitivity of wildlings to frost and freezing temperatures, but recurrent frost may also suppress flowering or it may damage the reproductive structures of plants. Likewise, *altitude* was found to affect negatively the proportion of reproductive wildlings, despite a short altitudinal range (30 to 307 m) in our sampling areas. Altitude may have acted as a surrogate of local edapho-climatic conditions as hypothesised by Catry et al. (2015). In fact, a posterior analysis revealed that the sampling areas at higher altitudes are also located towards inland, probably experiencing lower temperatures and higher thermal amplitudes, which may suppress flowering. Being in the *outside subplots* increased the chances of *E. globulus* wildlings being reproductive. This probably reflects lower competition for light and nutrients, which may enhance flowering (Williams et al. 2006), meaning that wildlings on the edges of plantations and those dispersed into adjacent open areas may be more likely to be reproductive. This may be corroborated by findings in Australia, where greater abundance of flowering *E. globulus* trees was found in the edges of plantations, when compared to the trees inside plantations (Barbour et al. 2008), and where lower tree densities in plantations promoted flowering in the related *E. nitens* (Williams et al. 2006). *Site index*, which is an indicator of site productivity, also increased the proportion of reproductive wildlings. It is probable that the same site conditions that enhance the growth of cultivated trees (nutrients, moisture), also favour the development of reproductive organs in younger plants.

4.4 Wildling establishment outside plantations

Survey transects outside plantations were established in different land cover types, indicating that *E. globulus* can spread into different habitats. *Land cover* effect was not analysed, because some land cover types were underrepresented. The unequal distribution of land covers could be indicative of different levels of susceptibility to invasion or an unequal representation of the areas surrounding *E. globulus* in the study region. Nonetheless, other studies found that some habitats such as native forests and

grasslands are more resistant to *E. globulus* invasion (Calviño-Cancela and Rubido-Bará 2013; Fernandes et al. 2016). In our study, most transects and most of the surveyed wildlings were established in *montado*, a common agroforestry system in the region characterised by an open forest of native oaks. Our survey protocol was biased towards finding *E. globulus* wildlings outside plantations, because the survey transects were triggered by the existence of at least one wildling at a minimum distance of 10 m. However, a recent study conducted in Central and North Portugal, using random survey transects outside *E. globulus* plantations, found wildlings in nearly one third of the transects (Fernandes et al. 2016), indicating that this is not a rare event.

In our study, we found *E. globulus* wildlings established up to a distance of 76 m, similarly to observations by Fernandes et al. (2016) in Portugal. These dispersal distances are consistent with the maximum seed dispersal distance from *E. globulus* plantations recorded in NW Spain (80 m; Calviño-Cancela and Rubido-Bará 2013). The sharp decrease in the number of *E. globulus* wildlings after 15 m was also observed in other studies investigating the escape of *E. globulus* (Larcombe et al. 2013; Fernandes et al. 2016) and other *Eucalyptus* spp. (Callaham Jr et al. 2013). Seeds of *E. globulus* do not have any particular adaptation for seed dispersal and most seeds are dispersed by gravity and wind (Cremer 1965; Cremer 1977). Calviño-Cancela and Rubido-Bará (2013) found that practically all *E. globulus* seeds (98.6%) fall within 15 m from the edge of plantations. Nevertheless, in this study, we found a larger proportion of wildlings at greater distances than in previous studies, which can be explained by our survey protocol. Instead of using survey plots along the transects outside plantations (e.g. Fernandes et al. 2016), we opted for a visual detection on both sides of the transect. As a result, we recorded more *E. globulus* wildlings than we would if we opted for survey plots. Our survey protocol should also explain why the proportion of adult *E. globulus* wildlings also increased with distance from the plantation, because larger wildlings are more easily detectable, while smaller wildlings likely remained unnoticed when distanced more than a few meters from the transect. It is important to stress that many of the surveyed wildlings established outside plantations possessed adult foliage, meaning that they overcame the most critical stage of survival and reached adulthood. This transition is recognised as a critical step in plant invasions (Richardson et al. 2000). Escaped wildlings can act as a

stepping-stone for posterior spread in the absence of management (Moody and Mack 1988; Richardson et al. 1994).

The factors influencing the distance of establishment of *E. globulus* from plantations highlighted the importance of the main vectors of seed dispersal: wind and water. Wind is considered the main vector of dispersal of eucalypts (Cremer 1977). The distance of wildling establishment was inferior in west exposure when compared to east, in line with the dominant winds in this region, which are predominantly from northwest. The same trend was observed in California, where the greatest dispersal distances of *E. globulus* wildlings coincided with the direction of the prevailing winds (McBride et al. 1988).

Water is also acknowledged as an important dispersal vector of eucalypt seeds (Kirkpatrick 1977; Forsyth et al. 2004). In our study, the presence of a natural drainage line was found to increase the distance of establishment of *E. globulus* from plantations suggesting that hydrochory may play an important role in wildling dispersal. In Australia, Larcombe et al. (2013) suggested that a drainage line might be responsible for the establishment of an *E. globulus* wildling at a distance of 175 m from a plantation. Apart from wind and water, ants may also contribute to the secondary dispersal of *E. globulus* seeds in Portugal (Deus et al. 2018b). Our analysis revealed that podzols affected negatively the distance of establishment from plantations. Soil itself has no influence on seed dispersal but it may affect wildling establishment. A study conducted in Portugal reported the opposite effect, finding that podzols positively influenced wildling abundance (Catry et al. 2015). We suspect that the effect of soil type maybe an artefact of the analysis related to other important factors such as slope and land cover.

5. Conclusions

The survey along the edges of *Eucalyptus globulus* plantations in Central Portugal provided evidence of an effective wildling recruitment since many wildlings overcame the most critical period for survival. The abundance of young *E. globulus* wildlings was mostly affected by soil cover variables symptomatic of the harmful impact of plant competition. Wildling presence was influenced by a wider range of site-scale factors including slope, aspect of the plantation edge and propagule pressure. Around 8% of the surveyed wildlings reached sexual maturity, supporting the hypothesis that the species is naturalised. A

few very young wildlings were reproductive suggesting that the species can have a very short juvenile period. The proportion of reproductive wildlings was related to different factors ranging from plant development (positively related to wildling size), plantation-related (positively related to stem age and site productivity) and climatic variables (negatively related to the number of frost days).

This study also provides evidence of an effective recruitment of *E. globulus* outside plantations, in different habitats, since more than half of the surveyed wildlings outside plantations were adult. Wildlings were found up to 76 m of distance. Greater dispersal distances coincided roughly with the direction of the prevailing winds and the presence of natural drainage lines.

This study tackled important knowledge gaps about the demography, phenology and ecology of *E. globulus* in the introduced range, which may help to identify the factors and situations enhancing *E. globulus* recruitment and escape from plantations. The development of reproductive wildlings can increase propagule pressure from plantation edges and outside plantations they can act as a stepping-stone for posterior spread in the absence of management. Particular caution is needed in downslope aligned with predominant winds and with the presence of drainage lines capable of significantly enhancing seed dispersal. Further research is needed to evaluate the reproductive capacity of *E. globulus* wildlings escaped from plantations, the parental relationships among naturally established populations outside plantations, the rate of expansion and the ecological succession of invaded areas.

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