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Combined effects of climate, habitat, and disturbance on seedling establishment of *Pinus pinaster* and *Eucalyptus globulus*

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Abstract The natural expansion of forestry trees into habitats outside plantations is a concern for managers and conservationists. We studied seedling emergence and survival of the two main forestry species in Portugal: *Eucalyptus globulus* (exotic) and *Pinus pinaster* (native); using a seed addition experiment. Our main objective was to evaluate the combined effects of climate (mild-summer and warm-summer climate), habitat (oak forest and shrubland), and disturbance (vegetation removal and non-disturbance) on the seedling establishment of species in semi- and natural habitats. Furthermore, we tested the effect of the "sowing season" (autumn and spring) on seedling emergence and survival. Overall, seedling establishment of both species was enhanced by light and water. However, we found important interactions among climate, habitat, and disturbance on both species' emergence and survival. The differences between habitats were more evident in the mild-summer climate than in the warm-summer climate. Our results also suggested that seedling survival may be enhanced by shrub cover in drier conditions (warm-summer climate). *Eucalyptus globulus* appears more sensitive to drought and disturbance changes than *P. pinaster*. In shrublands and mild-summer climate conditions, disturbance especially promoted *E. globulus* seedling establishment, while the forest canopy and the shade appeared to control it in both climatic conditions. After the first summer life, very low seedling survival was observed in both species, although the colonization of new areas appeared to be more limited for *E. globulus*. Our study suggests that climate conditions influence the effect (direction and intensity) of habitat and disturbance (plant–plant interactions) on seedling survival. Thus, the effect of light availability (forest canopy) and disturbance (vegetation removal) on these species establishment is climate context-dependent. This study presents very useful information to understand future shifts in these species distribution and has direct applications for the management of natural establishment outside the planted areas, and the management of the understorey to favor forest regeneration or limit forest colonization.

Keywords Biological invasions · Drought tolerance · Forestry species · Habitat suitability · Seedling survival · Portugal

Introduction

Forest plantations are one of the main causes of plant invasions worldwide (Richardson 1998). Nowadays,

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the natural expansion of forestry trees into habitats outside plantations is a concern for managers and conservationists, and a relevant issue for ecology research (e.g., Higgins and Richardson 1998; Richardson 1998; Richardson and Rejmánek 2004,2011; Pyšek and Richardson 2007; Dodet and Collet 2012; Richardson et al. 2014; Brundu and Richardson 2016). Among the most planted species worldwide, we can find *Eucalyptus* and *Pinus* species (Richardson 2011; FAO 2015), mainly due to the wide range of their ecological requirements, rapid growth, and high productivity (Richardson 2011). However, such characteristics and their widespread use in forestry can promote the invasion, by these species, of natural and seminatural habitats surrounding plantations (Essl et al. 2010; Procheş et al. 2012). Some prominent examples of invasive forestry trees species (and that have been particularly well studied) are species of the genera *Acacia* and *Pinus* (Richardson 1998; Rejmánek and Richardson 2013). The invasions by these species in South Africa threaten hundreds of native species with extinction, change fire and nutrient-cycling regimes, and water availability (Richardson et al. 1994; Higgins and Richardson 1998; Rejmánek and Richardson 2013). In order to contribute to a sustainable forest management, minimizing ecological risks, and maintaining economical productivity, it is crucial to understand the main factors that drive forestry species natural regeneration and to determine their establishment capacity outside their planted areas.

Natural regeneration comprehends several stages in the life cycle of plants, with seedling establishment recognized as a critical step for its success (Houle 1996; Castro et al. 2004; McAlpine and Jesson 2008). Young seedlings are more vulnerable to resource availability and stress factors, which in the long-term determine species establishment success and distribution (Niinemets and Valladares 2006). Indeed, seedling emergence and seedling survival capacity have been found to be related with invasive capacity (Niinemets and Valladares 2006; González-Muñoz et al. 2011; Calviño-Cancela and Rubido-Bará 2013). Understanding why some sites are more susceptible to the establishment of a given plant species than others has long been a central topic in biological invasions research. The establishment success of an exotic species depends on the characteristics of the new species itself and the host community, and on the interaction between them (Lonsdale 1999; Rejmánek et al. 2005; Richardson and Pyšek 2006), such that a species' colonization will not succeed in all recipient areas. In turn, susceptibility of a receiving community can change in function of competitive and facilitative interactions, nutrient availability, or disturbance levels (Davis et al. 2000). Particularly, the role of disturbance has been long since recognized as a possibly important driver of invasion (Elton 1958). For instance, many studies have observed that disturbance, specifically vegetation removal, favors exotic plants and can be a prerequisite for their establishment (e.g., Alston and Richardson, 2006; Davis and Pelsor, 2001; Davis et al. 2000; Higgins and Richardson, 1998; Mitchell et al. 2006).

In Portugal, forest plantations are predominantly composed of two species, *E. globulus* Labill. (exotic species) and *P. pinaster* Aiton (native species) (ICNF 2013). *Eucalyptus globulus*, native from Australia, was introduced in the middle of the 19th century, and since the middle of 20th century, the development of the pulp and paper industries originated the expansion of *E. globulus* plantation in Portugal (Alves et al. 2007). In turn, *P. pinaster* species is the most representative autochthonous species in Portugal, as confirmed by numerous archeological and geological evidences, for at least 33,000 years (Figueiral 1995). The current pine distribution area has been strongly influenced by human activitiy and since the 19th century has been used in large scale reforestation programs in most part of Mediterranean basin, often to control erosion (Barbéro et al. 1998), which lead to its expansion clearly beyond its natural distribution range (Figueiral 1995; Aguiar et al. 2007). Both species are highly represented in Portugal mainland, in which *E. globulus* representing 26% and *P. pinaster* 23% of the forest cover (ICNF 2013). These species also have similarities on geographical range, mostly occurring in the center and north of the country. Light and soil moisture conditions have been considered as the main drivers of plant distribution (Niinemets and Valladares 2006; González-Muñoz et al. 2011). Indeed, summer drought in the Mediterranean region is highly limiting for plant performance and recruitment (Chytrý et al. 2008). Accordingly, one of the major limiting constrains to *E. globulus* and *P. pinaster* natural establishment in the Iberian Peninsula is water availability (Almeida et al. 1994; Ruano et al. 2009; Rodríguez-García et al. 2010, 2011b; Alves et al. 2012; Catry et al. 2015). In addition, *P. pinaster* and *E. globulus* have been classified as pioneer species, requiring high light regimes to establish (Correia et al. 1989; Gil et al. 1990). In both pines and eucalypts, the seeds are found within hard structures (cones in the case of pines and capsules in eucalypts), and dispersion of seeds is influenced by wind speed and fire (Reyes and Casal 2001; Juez et al. 2014). However, the two species have very different seed dispersal abilities: *P. pinaster* seeds have large wings as a specialized wind dispersal structure and can be dispersed at long distances; *E. globulus* seeds have no adaptation for dispersal mechanism (wings or fleshy tissues). The differences in seed weight between species are remarkable; the average weight of the seeds (with coat) was 0.06 g in *P. pinaster* and 0.002 g in *E. globulus*. Furthermore, the thickness of the seed coat is clearly different, with *E. globulus* seeds having a thinner coat than those of *P. pinaster*. In *P. pinaster*, regular seed production initiates at 10–15 years old (in Iberian Peninsula) and large quantities of seeds are produced (Tapias et al. 2001). *Eucalyptus. globulus* sexual maturity occurs earlier than that of *P. pinaster*, usually at 3–4 years old, associated with the change to adult leaves (Jordan et al. 1999) and each capsule contains c. 6–25 viable seeds (Hardner and Potts 1995; Mimura et al. 2009). Both species' seeds germinate quickly whenever conditions are favorable (i.e., high water availability and light) (Reyes and Casal 1997, 2001).

In Portugal, given their exotic origin and their widespread use in forestry, *E. globulus* has been recently a controversial species in relation to the presence or not of the invasive behavior. The invasion risk of *E. globulus* was assessed in several publications based on the Australian Weed Risk Assessment (Daehler et al. 2004; Gassó et al. 2009; Gordon et al. 2012; Marchante et al. 2014). However, weed risk assessments (WRA) are mainly based on species biological traits and expert knowledge (Pheloung et al. 1999), and rarely based on field quantitative information (distribution of the species in the introduced range). Thus, given the extend of *E. globulus* plantation (high propagule pressure), their fast growth, the production of very large quantities of seeds, and in light of their diverse adaptations for dealing with disturbance (such as fire), the invasion risk of this species was classified as "High" (based on the Australian WRA) in Spain (Gassó et al. 2009), in Portugal (Marchante et al. 2014), in the USA (Gordon et al. 2012) in Hawaii, and other Pacific Islands (Daehler et al. 2004). In contrast with WRA classifications, field quantitative studies have reported *Eucalyptus* (including *E. globulus*) as a species with low invasive potential (da Silva et al. 2011; Callaham et al. 2013; Larcombe et al. 2013; Lorentz and Minogue 2015). In general, *E. globulus* has very seldom spread considerable distances from planting sites, and their natural establishment is frequently sporadic (Rejmánek and Richardson 2011; Larcombe et al. 2013; Fernandes et al. 2016). Understanding the factors that may reduce or improve *E. globulus* natural establishment should help in predicting the invasive potential of this species (Richardson and Rejmánek, 2011). In Portugal, the natural establishment of this species has been reported in the roadsides adjacent to plantations (Catry et al. 2015) and in burned plantations (Águas et al. 2014). However, the knowledge about their potential establishment from seeds and into different habitats and ecological conditions is still almost inexistent.

In turn, because of its commercial importance and easy acclimation, *P. pinaster* has been planted also in temperate regions outside its natural range. In fact, pine expansion is recognized as a global phenomenon (Richardson and Rejmánek 2004). Outside its natural range, *P. pinaster* and several other pines are counted among the most invasive plant species, mainly in the southern hemisphere (Richardson 1998; Richardson and Rejmánek 2004, 2011).

In Portugal, both species' plantations are surrounded by different natural and seminatural habitats, and their seeds are dispersed to a variety of microhabitats that provide a range of differences in biotic and abiotic conditions affecting seedling emergence, survival, and growth, hence influencing establishment success (Castro et al. 2004; Niinemets and Valladares 2006). In addition, establishment success may be related to seedling size as well as ability to withstand environmental stress and grow rapidly enough to capture resources during dry periods (Holmgren et al. 1997; Cavender-Bares and Bazzaz 2000). Rodríguez-García et al. (2011a) found that early-emerging *P. pinaster* seedlings had greater chances of establishment. Although the ecology and physiology of these species is well known (Correia et al. 1989; Porte and Loustau 1998; López et al. 2000; Schwanz and Polle 2001; Sabaté et al. 2002; Correia and Almeida 2004; Rodríguez-García et al. 2011a, b, c; Rodríguez-García and Bravo 2013), little is known about the interactive effects of climate, vegetation community, and disturbance on these species colonization capacity, or the habitat characteristics that hamper or favor these species establishment.

In the light of this, the aim of this study was to evaluate the combined effects of climate (mild-summer and warm-summer climate), habitat (oak forest and shrubland), and disturbance (vegetation removal and non-disturbance) on the seedling establishment (seedling emergence and survival) of *P. pinaster* and *E. globulus* in semi- and natural habitats. Furthermore, we tested the effect of the "sowing season" (autumn and spring) on seedling emergence and survival in order to evaluate the effect of seedling size on species ability to withstand environmental stress during summer. More specifically, we asked: (i) how does these factors affect the establishment success of these species (exotic and native species)? (ii) is the effect of habitat and disturbance dependent on climate conditions? (iii) is the effect of disturbance dependent on whether the seedlings are inside the forest or in the open field? This information is crucial to understand the response of these species to environmental conditions and can help us to explain future shifts in these species' distribution and natural establishment outside the planted areas.

Methods

Study sites

Field sites were established along the Atlantic coast in the northern (Aveiro, 48°37′N, 8°34′W, 21 m.a.s.l) and southern (Setúbal, 38°39′N, 8°36′W, 85 m.a.s.l.) of Portugal (in order to study two different climatic conditions) (Fig. 1). Mean annual temperature is 15.6 and 16.5 °C in Aveiro and Setúbal, respectively. Average (30 year average 1980–2010) annual precipitation is 944 mm in Aveiro (nearest meteorological station 40°38′N, 8°39′W). Setúbal site is drier with an average annual precipitation of 735 mm (nearest meteorological station 38°33′N, 8°53′W). At both sites, the precipitation occurs predominantly from autumn to early-spring (October–April) (Fig. 1). Thus, average annual precipitation in Setúbal is around 200 mm lower than in Aveiro. Climatic differences between sites are more evident during summer months (June to August), with drier and hotter summers in Setúbal (Fig. 1). Precipitation during summer months is very low in both sites (24 and 63 mm in Setúbal and Aveiro, respectively). In Setúbal, mean temperature during summer is 22.4 °C with a mean maximum temperature for the hottest month of 30 °C (August) (Fig. 1), whereas, in Aveiro, mean temperature during summer is 19.8 °C with a mean maximum temperature for the hottest month of 24 °C (August) (Fig. 1). The climate is Mediterranean with an oceanic influence and is classified in Aveiro as humid-temperate variant and in Setúbal as a sub-humid-warm variant, according to the pluvio-thermic coefficient of Emberger (Quezel 1977). According to the Köppen climate classification, Aveiro is classified as temperate climate with dry and mild summer (Csb), and Setúbal is classified as temperate climate with dry and warm summer (Csa). For simplicity purposes, we will refer to Aveiro and Setúbal climate conditions as mild-summer climate and warm-summer climate, respectively.

The vegetation is spatially heterogeneous in both sites, composed of herbaceous, shrub, and tree patches. For this study, two different vegetation communities (habitats) at each study site were selected: tree patches (forest habitat) and shrubland (see Experimental design). In the northern site, study was performed in a native pedunculated oak (*Quercus robur* L.) forest patch and in a near shrubland dominated by short shrubs (around 0.5 m height), predominantly gorse (*Ulex* spp.) and heath (*Erica* spp.), and also some herbaceous species. In the southern site, the study was performed in a cork oak woodland composed by *Quercus suber* L. and *Quercus ilex* L. (seminatural forest ecosystem "Montado") and in adjacent shrubland dominated by rockrose (*Cistus* spp.) and other Cistaceae (e.g., *Halimium* spp.) and gorse (*Ulex* spp.) These are the most common types of habitats adjacent to *E. globulus* and *P. pinaster* plantations. Plantations of *P. pinaster* and *E. globulus* are present in both sites, although no individual of these species was present within the areas of the experiment.

Experimental design

Our experiment was designed to evaluate the effect of disturbance as well as the particular effect produced by different climate conditions (mild-summer and warm-summer climate) and different habitat types (forest

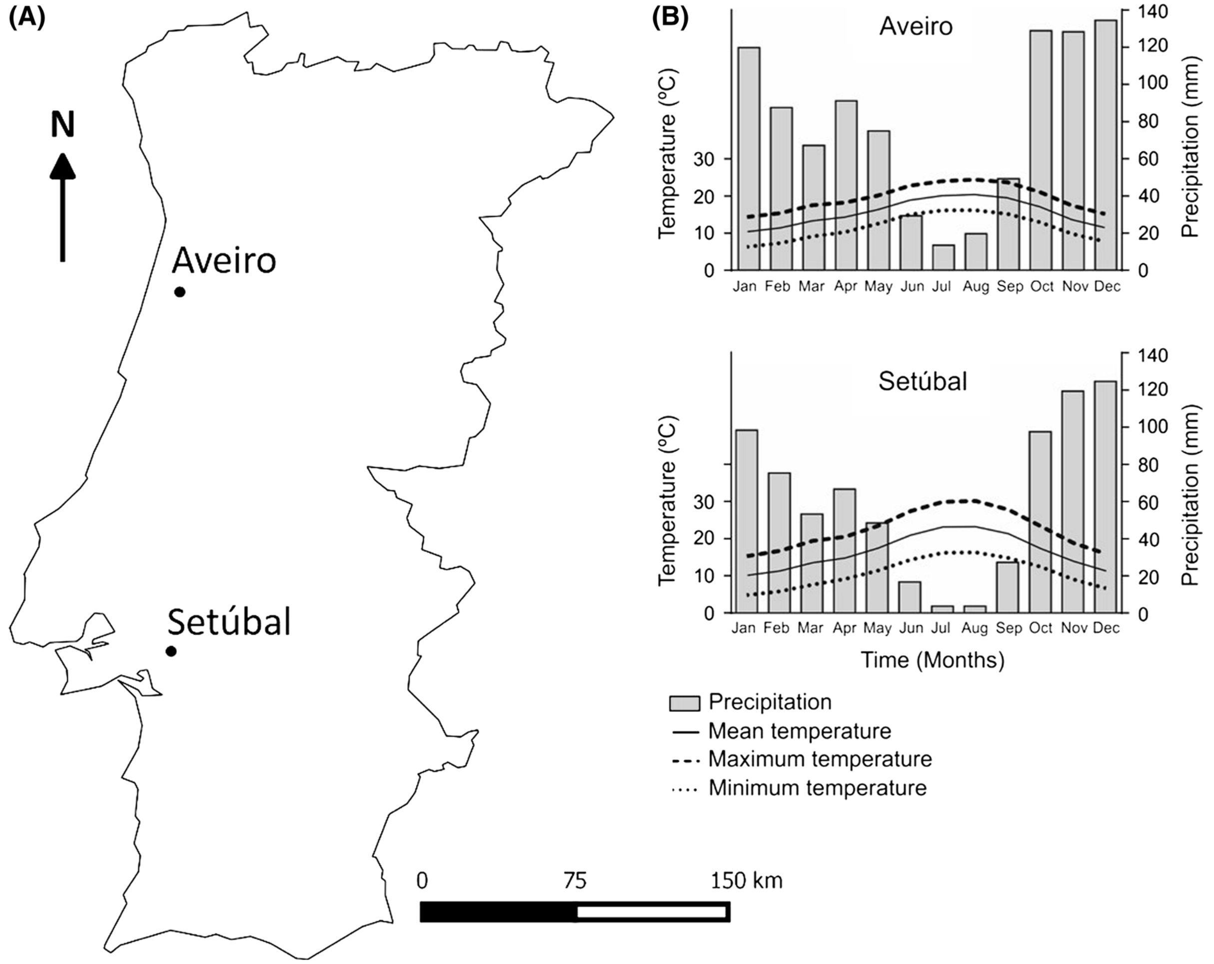

Fig. 1 Study sites location in Portugal (**a**), and climatic conditions (30 year average 1980–2010) of Aveiro (north) and Setúbal (south) study sites (**b**)

and shrubland) on *E. globulus* and *P. pinaster* seedling establishment. The potential for seedling emergence and survival of each species were evaluated through a seed addition approach similar to da Silva et al. 2011. In each of the two sites, 40 plots of 1 m^2 (randomly distributed) were set within the understory of the forests and in shrubland patches (see Study sites). In order to examine the effects of disturbance, plots were randomly left non-disturbed or disturbed through removal of the vegetation layer and soil scarification with a rake, with a total of 20 plots per disturbance treatment at each habitat type. Of these, ten plots were randomly selected for sowing seeds of each species. In order to avoid variation in seed viability within each species and between species, the seeds used in this study were commercial seeds with the maximum germination rate (99% in both species) under ideal conditions. In each plot, we sowed 60 *P. pinaster* seeds (approximately 4 g) or 150 *E. globulus* seeds (approximately 0.4 g) which were placed on the ground (randomly scattered) to simulate the conditions with which seeds are naturally dispersed. To test the effect of different sowing seasons on seedling emergence and survival percentage, seeds were sown in two different dates representing different seasons: spring (March 2013) and autumn (November 2013), with the experiment being replicated (new plots established) for each sowing season. A total of 320 plots were monitored (two species × two sites × two habitats × two disturbance treatments × two sowing seasons × ten plots). Plots were checked for seedling emergence and survival every 15 days in the first two months after sowing and monthly afterwards, until September 2013 for spring plots and September 2014 for autumn plots, respectively. Thus, the spring experiment lasted for 180 days and the autumn experiment lasted for 300 days.

Measurements of seedling emergence and survival

In each census, we used a grid with the same total area of the plot (1 m^2) subdivided in 10 × 10 cm squares in order to better record newly emerged seedlings at each counting date and distinguish them from new seedlings in subsequent counts (new seedlings were distinguished from survivors). In this way, the temporal duration of survival for each of the seedlings observed was determined based on when each seedling was first observed and when it was last observed (seedling death).

Data analyses

To calculate the final emergence percentage of each plot we used the ratio between the total number of emerged seedlings and total seeds sown. We also assessed the final survival percentage of each plot by calculating the ratio between the total number of seedlings that were still alive at the end of the study and the total number of emerged seedlings. We used GLM models (General Linear Model; factorial ANOVA) to determine the effects of sowing season (autumn or spring), site (mesic or xeric), habitat type (forest or shrubland), and disturbance treatment (disturbed or non-disturbed) on final seedling emergence and final seedling survival percentage for each species separately. Both sets of data (emergence and survival) were arcsine-transformed to meet assumptions of the analysis (normality and homogeneity of variances).

Multiple pairwise comparisons of means and differences between species were tested using Tukey HSD post hoc tests (Zar 1999). Means are reported with standard deviation (±SD).

Survival rates were analyzed using a time to event approach, i.e., we modeled the time that the seedlings remained alive (survival times). Seedlings that were still alive at the end of the study were considered censored data, as they may die or not in the future. This approach was also used in other studies (e.g., Calviño-Cancela and Rubido-Bará, 2013; Lorentz and Minogue, 2015; Wassie et al. 2009). The model used for the analyses was Kaplan–Meier survival analyses (Kleinbaum and Klein 2005) which estimated the survival functions (the probability of a seedling remaining alive at time t) for each factor group. Cox-regression survival analysis (Cox Proportional Hazards) was used to see the interactions among factors explicitly (Kleinbaum and Klein 2005).

All statistical analyses were performed with the STATISTICA 13.0 package (Dell Inc., Tulsa, OK, USA).

Results

Seedling emergence

We analyzed the effects of sowing season (spring vs autumn), climate (mild-summer climate vs warm-summer climate), habitat type (forest vs shrubland), and disturbance (with vs without vegetation removal) in seedling emergence percentage of each species separately (Table 1). The sowing season did not affect seedling emergence in both species (Table 1).

For *P. pinaster*, emergence percentage differed significantly between habitat types (Table 1). Specifically, seedling emergence of *P. pinaster* was more limited in the forest than in the shrubland habitat (8.50 ± 5.23 vs. 10.88 ± 6.68%, $N = 80$, $P = 0.0223$). However, we found no significant interactions among factors (Table 1; Fig. 2).

For *E. globulus* seedling emergence, we found a significant interaction between climate, habitat, and disturbance treatments (Table 1). Specifically, emergence was significantly higher in the site with a mild-summer climate than in the site with a warm-summer climate although only in the disturbed plots (Fig. 2). In addition, we observed that in both climate conditions, *E. globulus* seedling emergence was significantly higher in the shrubland than in the forest habitats (Fig. 2). In the mild-summer climate, emergence was higher in the disturbed than in the undisturbed plots, although only significantly different in the shrubland habitat (Fig. 2). In turn, in the warm-summer climate, we found no significant differences between disturbance treatments (Fig. 2).

Pairwise comparisons between species showed that *E. globulus* emergence percentage was significantly higher than *P. pinaster* ($F = 27.047$, $P < 0.001$) only

Table 1 GLM analyses of the effects of sowing season (spring, autumn), climate (mild-summer climate, warm-summer climate), habitat (forest, shrubland), and disturbance (disturbed, undisturbed) on seedling emergence percentage of *P. pinaster* and *E. globulus*

Effects	*P. pinaster*		*E. globulus*	
	F value	*P* value	*F* value	*P* value
(Intercept)	1348.819	<0.0001	2333.044	<0.0001
Sowing season	1.532	0.2179	0.373	0.5422
Climate	3.334	0.0699	**75.587**	**<0.0001**
Habitat	**5.219**	**0.0238**	**142.996**	**<0.0001**
Disturbance	0.015	0.9017	**5.665**	**0.0186**
Season × climate	0.016	0.8988	1.305	0.2552
Season × habitat	0.090	0.7643	0.346	0.5571
Climate × habitat	0.313	0.5766	1.273	0.2610
Season × disturbance	0.053	0.8187	0.938	0.3345
Climate × disturbance	0.204	0.6525	**21.748**	**<0.0001**
Habitat × disturbance	0.067	0.7959	0.757	0.3858
Season × climate × habitat	0.019	0.8915	1.267	0.2621
Season × climate × disturbance	0.037	0.8472	0.105	0.7468
Season × habitat × disturbance	0.306	0.5807	0.115	0.7349
Climate × habitat × disturbance	0.071	0.7901	**8.921**	**0.0033**
Season × climate × habitat × disturbance	0.715	0.3991	0.189	0.6645

Significant effects are highlighted in bold

in the shrubland habitat with a mild-summer climate conditions (Fig. 2).

Seedling survival

Pinus pinaster, seedling survival rate, examined with Kaplan–Meier survival analysis, showed a significant effect of season, climate, and habitat (Table 2). Specifically, we observed that the mortality rate was significantly higher in the spring than in the autumn sowing season (Fig. 3). Survival rate was significantly higher in the mild-summer than in the warm-summer climate (Fig. 3). In both climate conditions, seedling survival rate was significantly higher in the shrubland than in the forest habitat (Fig. 3). In addition, interaction between climate and disturbance treatment was also significant (Table 2). Survival rate was significantly higher in the disturbed plots than in the non-disturbed, although only in the site with a mild-summer climate (Fig. 3).

In relation to *E. globulus*, mortality was very intense in the first 2 months after emergence (Fig. 3). There was a significant interaction between sowing season and climate, between sowing season and habitat and, between climate and habitat (Table 2). Specifically, we observed that mortality rate was higher in the spring than in the autumn sowing season, although only significantly different in the mild-summer climate and in the shrubland habitat (Fig. 3). In addition, survival rate was significantly higher in the mild-summer climate than in the warm-summer climate, although only in the shrubland habitats (Fig. 3). Mortality rate was significantly higher in the forest than in the shrubland habitats (Table 2; Fig. 3). In forests, no survival was observed after 60 and 90 days since after emergence in the warm-summer climate and in the mild-summer climate, respectively (Fig. 3). The results also showed that climate, habitat, and disturbance treatment significantly interacted (Table 2). Specifically, in both habitat types in the mild-summer climate, survival rate was significantly higher in the disturbed than in the undisturbed plots (Fig. 3). In contrast, we found no significant difference between disturbance treatments in the forest habitat in the warm-summer climate but in the shrubland habitat, survival rate was significantly lower in the disturbed than in the undisturbed plots (Fig. 3).

For *P. pinaster* seedling survival percentage, we found a significant interaction between climate and habitat and between climate and disturbance treatments (Table 3). Specifically, we observed that survival percentage was significantly higher in the mild-summer climate than in the warm-summer climate,

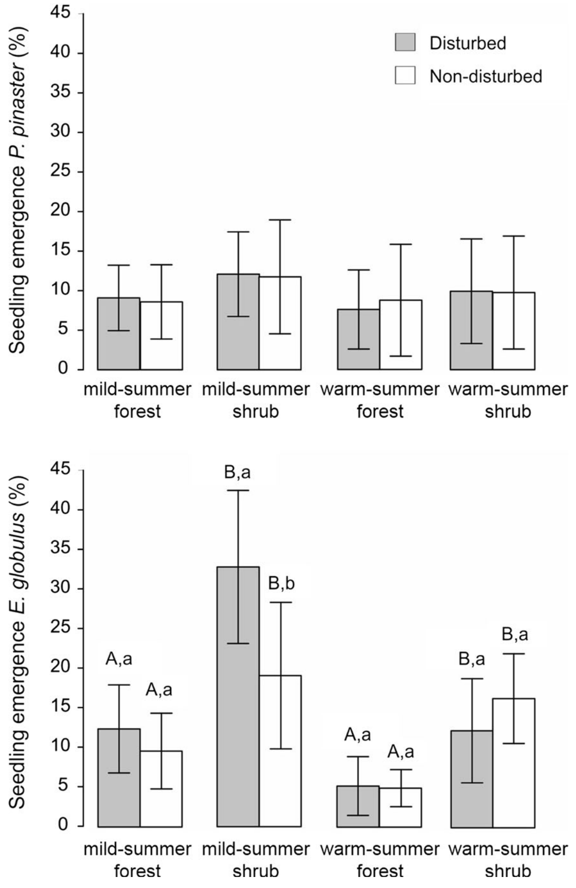

Fig. 2 Final seedling emergence percentage in *P. pinaster* (*upper graph*) and *E. globulus* (*lower graph*) species to all combinations of three factors: climate (mild-summer climate, warm-summer climate), habitat (forest, shrubland), and disturbance (disturbed, undisturbed). *Paired bars* depict mean ± 1SD under disturbed and undisturbed treatments, and climate and habitat are noted on the *x* axis. *N* for each *bar* = 20 (sowing seasons were merged). Different *uppercase letters* indicate significant statistical differences (Tukey Post hoc test, $P < 0.05$) between habitats within a single disturbance treatment at each study site, respectively. Different *lowercase letters* indicate significant statistical differences (Tukey Post hoc test, $P < 0.05$) between disturbance treatments within a single habitat

although only in the shrubland habitat and in the disturbed plots (Fig. 4). We found no significant differences between disturbed and non-disturbed plots within a single habitat at each study climate conditions (Fig. 4).

For *E. globulus,* surviving seedlings after the first summer of life were only found in disturbed plots in the shrubland habitat in the site with a mild-summer climate conditions (Fig. 4). We found no significant differences between sowing seasons (0.62 ± 1.52 vs. 1.47 ± 1.96% in spring and autumn seasons, respectively; $N = 10$, $P = 0.213$). Specifically, we registered a final survival of 3 seedlings of the 526 total emerged (0.6%) and 6 seedlings of the 459 total emerged (1.3%) after spring and autumn sowing, respectively.

Pairwise comparisons between species showed that *E. globulus* seedlings mortality rate was significantly higher than *P. pinaster* ($\chi^2 = 209.74$, $P < 0.001$) in both climate conditions and habitats, without influence of the sowing season (Fig. 3). Moreover, *P. pinaster* seedling survival percentage after the first summer of live was significantly higher than *E. globulus* ($F = 38.562$, $P < 0.001$) (Fig. 4). Specifically, we found significant differences between species in the shrubland habitat in the site with a mild-summer climate conditions and in the disturbed shrubland in the site with a warm-summer climate (Fig. 4). In total, we counted 74 *P. pinaster* seedlings (7.96%) and 9 *E. globulus* seedlings (0.27%) alive after the first summer of life.

Discussion

In this study, we tested the effect of the “sowing season” (autumn and spring) on seedling emergence and survival in order to evaluate the effect of seedling size on species ability to withstand environmental stress during summer. The results show that the mortality rates of *P. pinaster* and *E. globulus* seedlings were significantly affected by “sowing season”: the younger seedlings (from March to September) were more likely to die than older seedlings (from November to September). These results agree with results of Rodríguez-García et al. (2011a,c) and , who found that early emerging *P. pinaster* seedlings had greater chances of establishment than older seedlings. They propose that these differences in mortality rate are likely related to seedling size as older seedlings have increased ability to withstand environmental stress and grow enough to capture resources during summer. Nevertheless, after the first summer of life, no significant differences on seedling survival percentage were observed between sowing seasons. Summer drought is one of the most limiting factors for natural establishment of tree seedlings in Mediterranean systems and periods with high temperatures and drought accounted for high seedling mortality (many

Table 2 Kaplan–Meier survival analyses of the effects of sowing season (spring, autumn), climate (mild-summer climate, warm-summer climate), habitat (forest, shrubland), and disturbance (disturbed, undisturbed) on seedling survival rate of *P. pinaster* and *E. globulus*

Effects	D.F.	*P. pinaster*		*E. globulus*	
		χ^2 value	*P* value	χ^2 value	*P* value
Sowing season	1	**59.19**	**<0.001**	**13.98**	**<0.001**
Climate	1	**48.13**	**<0.001**	**74.53**	**<0.001**
Habitat	1	**41.08**	**<0.001**	**238.7**	**<0.001**
Disturbance	1	1.08	0.298	**35.38**	**<0.001**
Season × climate	3	104.9	0.027	**114.8**	**<0.001**
Season × habitat	3	103.8	0.521	**244.5**	**0.004**
Climate × habitat	3	89.71	0.664	**320.7**	**<0.001**
Season × disturbance	3	60.99	0.194	57.11	0.102
Climate × disturbance	3	**55.34**	**0.007**	**178.6**	**<0.001**
Habitat × disturbance	3	42.27	0.808	272.5	0.158
Season × climate × habitat	7	151.1	0.301	364.2	0.334
Season × climate × disturbance	7	118.5	0.249	218.6	0.515
Season × habitat × disturbance	7	105.8	0.637	299.2	0.070
Climate × habitat × disturbance	7	97.63	0.567	**410.7**	**0.003**
Season × climate × habitat × disturbance	15	168.0	0.426	456.6	0.655

Significant effects are highlighted in bold

seedlings became brown and dried out during the summer). These results can suggest that the difference in size between the younger ("spring sowing") and the older seedlings ("autumn sowing") was not enough to have a significant effect on their survivability during summer.

From seedling emergence phase to establishment phase (survival), air temperatures and water availability decreased, and climatic differences between sites became more accentuated during the summer months. Thus, water availability became more limiting as the experiment progressed, mainly in the site with a warm-summer climate conditions. The results of this study show (as expected) that natural emergence and survival of *P. pinaster* and *E. globulus* seedlings were significantly better in mild-summer climate conditions. Results obtained by other studies point to the same direction, showing that high soil moisture favored *E. globulus* and *P. pinaster* establishment (López et al. 2000; Rodríguez-García et al. 2011b; González-Muñoz et al. 2011; Catry et al. 2015). Along with water availability, light exposure has also been considered as an important driver in both species' establishment. *P. pinaster* and *E. globulus* have been classified as pioneer species, requiring high light regimes to establish (Correia et al. 1989; Gil et al. 1990). Forests patches dominated by pedunculated oak, like other broad leaved trees, have low light irradiance regimes at ground level compared to the open vegetation of shrubland (Barbier et al. 2008). Our results show that natural emergence and survival of *P. pinaster* and *E. globulus* seedlings were worst under forest canopy, although only in the mild-summer climate. These results agree with results of Calviño-Cancela and Rubido-Bará (2013), who observed lower *E. globulus* seedling emergence and survival under pedunculated oak forest than in shrubland. Sánchez-Gómez et al.(2006a), showed that *P. pinaster* has poor survival at lower irradiance levels. However, other studies have shown that *P. pinaster* establishment can be better under canopy cover than in the open (Ruano et al. 2009; Rodríguez-García et al. 2011a). Rodríguez-García et al. (2011a) suggest that milder conditions provided by overstorey canopy cover (higher relative humidity and lower air and soil temperature) may have protected seedlings from stressful environmental conditions. Our results also suggest that in the warm-summer climate, the effect of higher light availability in the shrubland was not significant on *P. pinaster* seedling survival. These results show that climate conditions can influence the effect of canopy cover on seedling establishment of these species. Considering the disturbance effects, it was expected that conditions after disturbance, with reduced intraspecific competition (higher water, light and nutrients availability), would enhance seedling

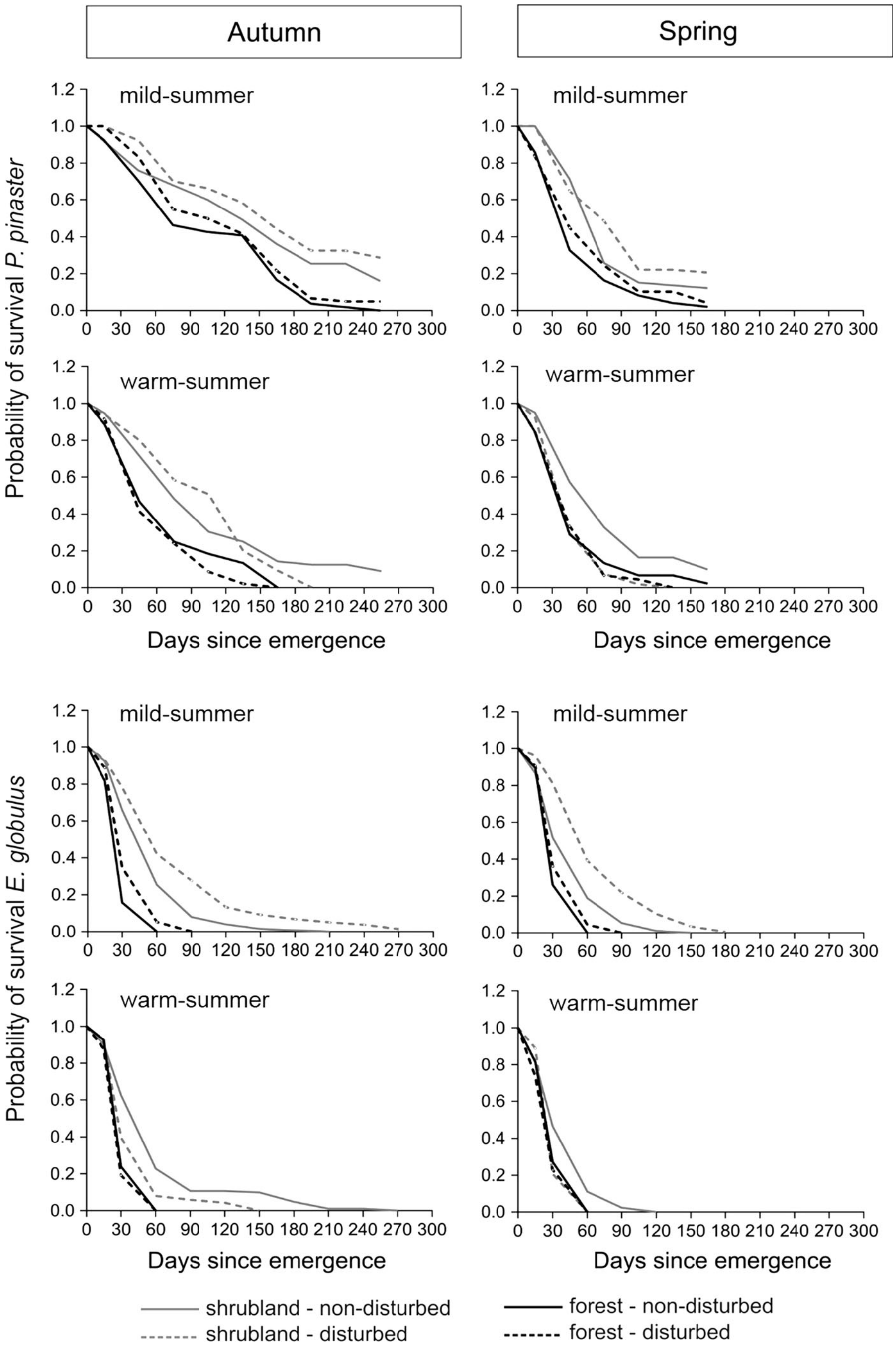

Fig. 3 Survival curves (Kaplan–Meier survival analysis) of *P. pinaster* and *E. globulus* seedlings emerged from each sowing season (autumn and spring) in the two climates (mild-summer climate and warm-summer climate) and habitats studied (forest and shrubland). Curves show the percentage of seedlings still surviving at different days since emergence in the disturbed and non-disturbed plots at each habitat within each site

emergence and survival (Castro et al. 2004). Microsites created by disturbance can differ greatly in levels of environmental resources and can, therefore, have a major influence on the establishment of younger seedlings. However, *P. pinaster* emergence and survival was not significantly affected by disturbance. In agreement with our results, others studies showed that shrub cover did not compete with *P. pinaster* seedlings (Calvo et al. 2003; Rodríguez-García et al. 2007, 2010, 2011b) and under drought stress, processes of facilitation may be more frequent that competition (Rodríguez-García et al. 2011a, c).

Table 3 GLM analyses of the effects of sowing season (spring, autumn), climate (mild-summer climate, warm-summer climate), habitat (forest, shrubland), and disturbance (disturbed, undisturbed) on percent survival of *P. pinaster* at the end of study

Effects	*F* value	*P* value
(Intercept)	63.756	<0.0001
Sowing season	0.0978	0.7549
Climate	**28.169**	**<0.0001**
Habitat	**36.897**	**<0.0001**
Disturbance	0.065	0.7997
Season × climate	0.186	0.6673
Season × habitat	0.243	0.6230
Climate × habitat	**13.823**	**0.0003**
Season × disturbance	0.555	0.4576
Climate × disturbance	**8.593**	**0.0039**
Habitat × disturbance	0.336	0.5633
Season × climate × habitat	0.084	0.7723
Season × climate × disturbance	0.393	0.5318
Season × habitat × disturbance	0.023	0.8793
Climate × habitat × disturbance	3.158	0.0777
Season × climate × habitat × disturbance	0.003	0.9596

Significant effects are highlighted in bold

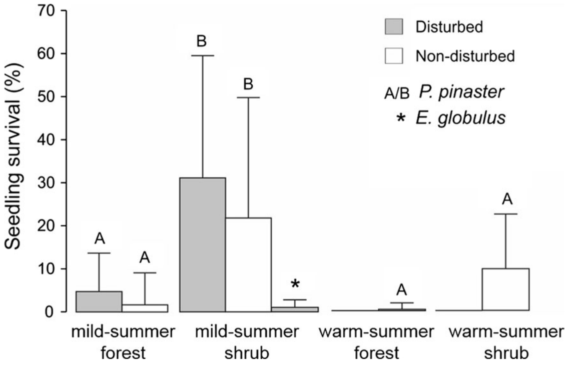

Fig. 4 Seedling survival (% of living seedlings per plot at the end of the study) of *P. pinaster* and *E. globulus* species in each type of disturbance treatment and habitat type at each study site. *Bars* depict mean ± 1SD under disturbed (*gray*) and undisturbed (*white*) treatments, climate (mild-summer climate and warm-summer climate), and habitat type (forest and shrubland) are noted on the x axis. N for each *bar* = 20 (sowing seasons were merged). Different *letters* indicate significant statistical differences (Tukey Post hoc test, $P < 0.05$) on *P. pinaster* survival between habitats within a single disturbance treatment at each study site. In all cases, the differences between disturbed and non-disturbed plots are no significant. *E. globulus* living seedlings at the end of the study were only registered in disturbed shrubland in mild-summer climate and their bar is marked by *asterisk*

Indeed, in habitats considered stressful for a target plant, host plant communities could facilitate the former's establishment through an amelioration of soil drought and high temperatures (Becerra and Bustamante 2011). This positive plant–plant interaction may also explain why the removal of vegetation did not benefit *P. pinaster* seedlings survival. Therefore, we argue that *P. pinaster* seedlings may benefit from the shading in drier conditions. Moreover, this species has enormous ecological plasticity, tolerates conditions varying from Mediterranean to Temperate–Oceanic climates (Rodríguez-García et al. 2011b). Indeed, *P. pinaster* can be more drought resistant than oak species (Sánchez-Gómez et al. 2006b). From the findings in our study, *P. pinaster* can also be more drought resistant than *E. globulus*. Thus, we have two factors that may have allowed *P. pinaster* establishment under drier conditions: its drought resistance and the facilitative effect of host plant communities. On the other hand, we observed a positive effect on *E. globulus* establishment by removing the vegetation layer, although only in the shrubland habitat in the mild-summer climate. Many studies also indicate that disturbance, specifically vegetation removal, favors exotic plants and could be a prerequisite for their establishment (e.g., Alston and Richardson, 2006; Davis and Pelsor, 2001; Davis et al., 2000; Higgins and Richardson, 1998; Mitchell et al., 2006). High mortality during initial phase of seedling growth due to unsuitable environmental conditions or competition with other plants has been mentioned by other studies as one of the most important factors that limits eucalyptus colonization (Rejmánek and Richardson

2011; da Silva et al. 2011; Callaham et al. 2013). Therefore, we argue that competition with other plants significantly limits *E. globulus* establishment in the mild-summer climate: higher mortality rate in the non-disturbed than in the disturbed plots. In contrast, mortality rate was significantly higher in the disturbed than in the non-disturbed plot in the warm-summer climate. This is in agreement with the described facilitative effect of host plant communities (amelioration of soil drought and high temperatures) in habitats considered stressful for a target plant (Bertness and Callaway 1994; Holmgren et al. 1997; Prider and Facelli 2004; Becerra and Bustamante 2011). Thus, our results suggest that mechanisms by which disturbance affected the *E. globulus* seedling emergence and survival depends on the climate conditions. In spite of the higher seedling emergence, *E. globulus* displayed higher mortality rate during the initial phase of seedling growth than *P. pinaster*. Moreover, we found surviving *P. pinaster* seedlings at the end of the study (established) in both sites and under both habitat types (12.4 and 2.8% mild-summer climate and warm-summer climate, respectively). In contrast, all *E. globulus* emerged seedlings died, except in the disturbed shrubland plots at the site with a mild-summer climate although with a very low survival percentage (0.27%). The larger seeds of *P. pinaster* originated robust seedlings in comparison with very tiny seedlings emerging from *E. globulus* seeds. Likewise, Reyes and Casal (2001) showed that larger seeds give rise to more vigorous seedlings and have a lower mortality rate than seeds of smaller size (Reyes and Casal 2001). *Eucalyptus* produce very large quantities of very small seeds with no obvious endosperm and the newly emerged seedlings (sustained by cotyledon photosynthesis) require that their roots penetrate quickly into suitable wet substrate to ensure survival (Rejmánek and Richardson 2011). Our results seem to indicate that *E. globulus* seeds can successfully emerge when water availability is adequate, but seedlings' survival and subsequently establishment are dependent on seasonal fluctuations in water availability during the establishment phase. Indeed, this species is also reported as highly sensitive to drought, particularly at the seedling stage when seedlings are especially vulnerable (Silva et al. 2004). Therefore, water deficit can compromise summer survival of young eucalyptus (Jacobs 1955; Stoneman 1994). Thus, *E. globulus* seedling establishment may be limited by the combination of two factors: (1) small seed size; (2) high vulnerability to drought.

In conclusion, we found important interactions among climate, habitat, and disturbance on both species' emergence and survival. The effect of forest canopy cover was more evident in the mild-summer climate than in the warm-summer climate. Our results also suggested that seedling survival may be enhanced by shrub cover in drier conditions (warm-summer climate). The effects of light availability and disturbance on these species establishment are climate context-dependent and cannot be generalized. These results may help to assess the impact of environmental conditions on these species establishment, and can help us explain future shifts in these species distribution and natural establishment outside the planted areas. Future climatic change scenarios, with droughts increasingly longer and more intense and climate irregularity (IPCC 2007), will affect these forest species establishments with more drastic effect on *E. globulus.*

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