Forest Ecology and Management 374 (2016) 129–135

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Assessing the invasive potential of commercial *Eucalyptus* species in Brazil: Germination and early establishment

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ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 2 May 2016
Accepted 3 May 2016
Available online 11 May 2016

Keywords:
Alien species
Invasiveness
Environmental conditions
Seed germination
Substrate water potential
Air moisture

ABSTRACT

The invasive potential of a species is related to the interaction between genetic and environmental characteristics, as climatic conditions and native vegetation, reflecting the species adaptation to local conditions. An ecologically-adapted alien species can alter diversity and structure of forest ecosystems. This study assessed the invasiveness potential of eight commercially important *Eucalyptus* species in the germination and early establishment phases in southeastern Brazil. The first experiment evaluated seed germination under controlled temperature and air humidity conditions testing six substrate water potentials. The second experiment was conducted in pots to evaluate seed germination and seedling mortality under rainfed and irrigated regimes. Nine field trials were set up in the third experiment in six locations. In each trial, 80 plots of 1 m^2 were installed with half the plots undisturbed and half where preexisting plants and litter were removed. In the first experiment, *Eucalyptus* taxa differed significantly in seed germination for substrate water potential $\geqslant -0.6$ MPa. No germination was found for any taxa at -0.8 MPa substrate water potential. In the second experiment, differences in germination between species and water treatments were observed 21 days after sowing. No mortality was found in the irrigated treatments. No seedling mortality was observed in the rainfed treatments when the relative air humidity was higher than 85%, even during a dry period of 45 days. By contrast, seedling mortality was observed for all taxa in all the rainfed treatments when relative air humidity fell under 80%. In the third experiment, germination and seedling establishment of *Eucalyptus* species was always very low. The non-*Eucalyptus* species showed differences in germination between trials and soil exposures. Our results showed that soil and air moisture were major drivers of eucalypt germination and initial establishment in controlled conditions. However, under field conditions additional factors such as competition of non-*Eucalyptus* species and seedlings predation drastically limited eucalypt establishment suggesting low ecological adaptation of *Eucalyptus* sp. as an invasive species.

1. Introduction

Eucalypts are used in commercial forest plantations due to their high growth rates, adaptability to various ecological conditions (e.g. elevation, climates, soils) and multiple uses (e.g. raw material, energy wood, timber, pulp and paper). In recent decades, significant increases in productivity have been obtained through *Eucalyptus* taxa selection and breeding, plant protection and management practices (Gonçalves et al., 2013). However, there are concerns about the environmental impacts of commercial eucalypt stands (Silva et al., 2014). One major concern is eucalypt invasion which may happen in any ecosystem when favourable conditions occur (Larcombe et al., 2013; Tererai et al., 2013). Few studies have been carried out to assess the invasive potential of seedlings of *Eucalyptus* species from planted stands into native forests either in its native range in Australia (Larcombe et al., 2013) or in exotic environments such as the United States (Booth, 2012; Gordon et al., 2012; Callaham et al., 2013; Lorentz and Minogue, 2015) or Brazil (Silva et al., 2011).

The invasiveness of a species is a consequence of the interaction between genetic and environmental characteristics reflecting the adaptation of that species to local conditions (Godfree et al.,

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http://dx.doi.org/10.1016/j.foreco.2016.05.007
0378-1127/

2004; Erfmeier and Bruelheide, 2010). Temperature, air and soil humidity are usually key factors for seed germination and seedlings establishment (Kozlowski, 2002; Mc Carragher et al., 2011; Stanton-Geddes et al., 2012) as observed for soil water potential for *Eucalyptus* species (Arnold et al., 2014). However, these factors may vary between species. An ecologically-adapted alien species may cause habitat loss of endangered species and alter genetic diversity and forest ecosystems structure (Gurevitch and Padilla, 2004; Williams and Wardle, 2007). Species with a broader tolerance of environmental conditions could present higher potential risk of invasion than less tolerant species (Higgins and Richardson, 2014).

Nine *Eucalyptus* species are used in more than 90% of the current plantations (Harwood, 2011). Eucalypts have been planted extensively beyond their natural range in more than 90 countries and have become invasive in a few places (Mattei and Longhi, 2001; Forsyth et al., 2004; Ruthrof, 2004). According to Gordon et al. (2012), some widely used *Eucalyptus* species of the *Symphyomyrtus* subgenus (*Eucalyptus globulus* Labill., *Eucalyptus camaldulensis* Dehnh., *Eucalyptus grandis* W. Hill ex Maiden, *Eucalyptus saligna* Sm. and *Eucalyptus urophylla* S.T. Blake) present potential risk of becoming invasive in the USA. It is therefore important to get insights into the conditions that affect germination and early establishment of the seedlings of these species to ensure appropriate management of commercial stands. This issue can be addressed in field experiments established across appropriate ecological ranges (Flory et al., 2012) in regions where *Eucalyptus* plantations are already established as well as in areas where commercial companies intend to deploy new plantations (Richardson and Rejmánek, 2011).

This study was conducted in six regions in Brazil under different climatic/ecological conditions with five pure species (*E. camaldulensis*, *Eucalyptus pellita* F. Muell, *E. grandis*, *E. urophylla*, *E. saligna*) and three hybrids (*E. urophylla* × *E. grandis*, *E. globulus* × *E.* spp., *Eucalyptus dunnii* Maiden × *E.* spp.) of eucalypts. This work extended that of Silva et al. (2011) dealing with the invasiveness potential of seedlings from *E. grandis* and hybrid of *E. urophylla* × *E. grandis* clones in three regions in Brazil.

A key determinant of invasion potential is the ability for species to reproduce effectively in a novel environment (Perkins et al., 2011; Booth, 2015). Understanding the effect of environmental factors such as substrate moisture and air humidly on germination, and ecological factors such as competition with native species, will therefore provide clues to the invasive potential of introduced species. Two questions were therefore addressed in the present study: how do substrate moisture and air humidity affect seed germination and seedling survival of *Eucalyptus* species? How do seed germination and early establishment under controlled and uncontrolled conditions differ among *Eucalyptus* species?

2. Materials and methods

Three experiments were set up in various locations and ecological conditions. We selected eucalypts that are among the most planted species worldwide (Harwood, 2011), whose seeds are produced in Brazil. The seedlots were bulks of open pollinated seeds collected from at least 12 trees in a seed orchard for each species. The same seedlots were used in the three experiments (Table 1). For a given species, germination rate of 100%, considered as reference seedling germination in experiments one and two, was estimated by counting the number of seedlings 14 days after sowing one gram of seeds + chaff in controlled conditions, as described in the experiment one with substrate water potential of 0 MPa.

The first experiment aimed at evaluating the germination of *Eucalyptus* species seeds under controlled conditions of temperature (25 °C) and air humidity with substrate water potentials (Ψ_s) of 0.0, −0.2, −0.4, −0.6, −0.8, and −1.0 MPa. The water potential control was achieved by adding a polyethylene glycol 6000 solution to a paper substrate (Villela et al., 1991). For each taxon, five replicates of 0.1 g seed were sown for each value of substrate water potential. Seedlings were counted at 14 days after sowing (DAS). The experiment was conducted in the laboratory of Sementes e Melhoramento Florestal (Seeds and Forest Improvement) – UNESP Jaboticabal (latitude: 21°08S; longitude: 48°11W; elevation: 583 m a.s.l.).

The second experiment was conducted at the IPEF nursery in Piracicaba, São Paulo State, Brazil, (Koppen classification: Cwa, latitude: 22°25′S; longitude: 47°22′W; elevation: 554 m a.s.l.; mean annual temperature: 21.6 °C; mean annual precipitations: 1260 mm). A complete randomized design was set up with 16 treatments (8 species × 2 water regimes) and four blocks. This experiment aimed at evaluating the germination of *Eucalyptus* seeds and the initial establishment of seedlings under rainfed condition or irrigated with 5 mm of tap water, three times a week. For each taxon, 1 g of seed was sown in pots of 50 dm^3 filled with river sand. The number of seedlings was counted at 6, 7, 10, 13, 15, 17, 21, 29, 38, 55, 107 and 140 DAS. Rainfall, temperature and relative air humidity were measured daily during the experiment period.

The third experiment aimed at evaluating under field conditions the establishment of seedlings of the different eucalypt species/hybrids. Nine trials were set up in six locations (Table 2), during spring and summer when high precipitations occur and the *Eucalyptus* species studied have viable seeds in southeast Brazil. (I. Vieira, IPEF Seeds Coordinator, personal communication). In each trial, 80 plots of 1 m^2 were established with 40 plots where the soil and vegetation was undisturbed (hereafter referred to as "soil unchanged" – SU) and 40 plots where plants and soil litter were removed (hereafter referred to as "bare soil" – BS). In each plot, 1 g of seeds was sowed, representing a potential of >200 seeds m^{-2}. A split-plot design with five blocks was set up with taxon as whole plot factor and soil exposure (SU or BS) as subplot factor. The trial was replicated one year after the first sowing in three locations in order to check possible variations between years in seedling germination and seedling establishment. The plots were assessed at approximately 10-day intervals for at least 40 DAS. The number of eucalypt seedlings that had germinated

Table 1
Studied eucalypts taxa and seed lot provenances.

Code	Species	Section	Provenance
1	*E. camaldulensis*	Exsertaria	Selvíria, MS (SPA-F1)
2	*E. dunnii* × *E.* spp.[a]	Maidenaria	Itatinga, SP T6 H27 (SPA-F2)
3	*E. globulus* × *E.* spp.[a]	Maidenaria	Camanducaia, MG (SPA-F1)
4	*E. grandis*	Latoangulata	Lençóis Pta., SP T16601 (SPA-F2)
5	*E. saligna*	Latoangulata	Itatinga, SP T20 (SPA-F1)
6	*E. pellita*	Latoangulata	Anhembi, SP T19C113 (SPA-F1)
7	*E. urophylla* × *E. grandis* (*E. urograndis*)	Latoangulata	Itatinga, SP T23A079 (SO-F2)
8	*E. urophylla*	Latoangulata	Piracicaba, SP 02AV01 (SPA-F2)

[a] Pure species are not found in the areas of seed production. MS: Mato Grosso do Sul state; SP: São Paulo state; MG: Minas Gerais state; SPA: Seeds Production Area; and SO = Seed Orchard.