



Do chill hours and soil moisture limit the germination of *Elaeagnus angustifolia*?

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Abstract

Invasive exotic species produce impacts of different magnitudes in the environment. The colonization success of a species depends on several factors. Different research demonstrated that chill hours accumulation is one of the most important factors affecting the germination of the tree species *Elaeagnus angustifolia* L. This non-native species is considered invasive, even noxious, in different parts of the world, modifying terrestrial and aquatic environments. We investigate the chill hours accumulation effect on the seed germination of *E. angustifolia* under two soil moisture conditions (*field capacity* and *half-field capacity*). We also evaluated the survival of seedlings of the species for 240 days under the two mentioned moisture conditions. With the data obtained, we made two heat maps (one for each moisture condition) with the probability of germination of *E. angustifolia* in Argentina related to the natural availability of chill hours. We found that as the chill hours accumulation increased, germination of *E. angustifolia* increased in half-field capacity treatment and decreased in field capacity conditions. Seedling survival was 100% in both water regimes. Heat maps show the break dormancy probability of *E. angustifolia* seeds throughout the Argentine territory, with high potential of germination in Patagonia. In addition to contributing to the prevention of the invasion of *E. angustifolia* in Argentina, the information generated in our study can be helpful in other regions of the world since the significant genetic variability that the studied species presents can make its environmental distribution range unpredictable.

Keyword Russian olive · Chilling requirements · Germination potential

Introduction

Exotic species distribution has been molded, in part, by human activity (Kuebbing and Nuñez 2016), but mechanisms that allow some species to become invasive are unclear (Bajwa et al. 2016; Warren et al. 2019). However, several factors can limit the establishment success of alien

species, e.g., environment, biotic, and dispersal (by humans or nature) (Theoharides and Dukes 2007; Richardson and Pyšek 2012). The effect of these limiting factors is stronger at the ecological niche boundaries of the invasive species under extreme conditions (e.g., extreme cold or heat) (von Holle 2013). These findings contribute to the understanding of relationships between environmental variables and species distribution patterns, which is a useful conservation planning tool for natural ecosystems (Jeganathan et al. 2004; Young 2007).

Temperature and soil water availability are some of the most important environmental factors affecting seed dormancy and germination (Lambers and Oliveira 2019). Temperature affects the percentage of germination through the physiological relationship between temperature and latency (Wang and Berjak 2000; Chuine and Beaubien 2001; Morin et al. 2007; Bonner 2008). In addition, the germination requirements of seeds partially determine the distribution of plant species in space and time (Fenner 1980; Venable and Lawlor 1980). Tree species from temperate zones usually

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have seed dormancy (Baskin and Baskin 2014) that naturally needs chill hour accumulation to break it. So, a cold stratification period is needed in germination experiments, ideally between 0 and 7 °C (Cesaraccio et al. 2004; Richardson et al. 1974). The chill hours accumulation is the sum of hours in that the temperature is under a base temperature which varies depending on the species (Batlla et al. 2009; Scholten et al. 2009). Several exotic tree species contribute to landscape homogenization, reduce native biodiversity, and modify ecosystem functionality in undesirable directions (Gaertner et al. 2011; Cardinale et al. 2012; Gamfeldt et al. 2013) leading to a profound physiognomic change. This is especially problematic in environments that naturally lack or have few woody species, such as grasslands and savannas, so the impacts of the invasion are greater (Huxman et al. 2005; Rolhauser and Batista 2014; Archer et al. 2017).

Elaeagnus angustifolia L. is a tree native to Europe and Central Asia that has become naturalized in different temperate regions of the world and forms monospecific strata on the riverbanks (Klich 2000). The species presents a large variability of germination rates (23 to 83%) that differ across individuals (Kiseleva and Chindyeva 2011). Previous studies have shown that *E. angustifolia* can germinate in both light and shade conditions (Reynolds and Cooper 2010; Shafroth et al. 1995) and can grow in flooded areas or under drought conditions in its native distribution range (Stannard et al. 2002; Asadiar et al. 2012) and in its introduced range (Katz and Shafroth 2003; Reynolds and Cooper 2011). The species is found from sea level to more than 2000 m high and tolerates a wide range of climatic and edaphic conditions (Dubovyk et al. 2014), both in its natural range (Stannard et al. 2002; Asadiar et al. 2012) and introduced (Katz and Shafroth 2003; Reynolds and Cooper 2011). Zhang et al. (2018) found that the thresholds of the annual mean temperature (AMT) of highly suitable habitats for *E. angustifolia* were 1.8 and 15.4 °C. Other studies suggest that it can resist a range of temperatures from -45 to 45 °C, flooded areas or under drought conditions, high salinity, and alkalinity in soils (Wang et al. 2006). *Elaeagnus angustifolia* can colonize disturbed sites (Dubovyk et al. 2014) since its seeds can persist in soil seed banks (Klich 2000; Brock 2003) and, in addition, it has the ability to reproduce vegetatively from stems and roots (Stannard et al. 2002; Klich 2013) allowing it to establish itself and invade new areas. The additional moisture (promoted by riverbanks, plains, and reservoir margins or irrigation channels) facilitates the distribution and abundance of *E. angustifolia* (Nagler et al. 2011). Several researches on *E. angustifolia* germination agree that cold stratification positively affects the proportion of germinated seeds (Hogue and LaCroix 1970; Olson 1974; Hamilton and Carpenter 1975; Olmez et al. 2007; Kiseleva and Chindyeva 2011; Guilbault et al. 2012). In particular, Guilbault et al. (2012) found that seeds with the most extended period

of cold stratification treatment showed the highest germination percentage, while the lowest percentage was from the treatment without cold stratification. From these results, the authors set a geographical limit of distribution of the species in North America imposed by the insufficient accumulation of chill hours necessary to break the dormancy of the seeds. Nevertheless, habitat modeling is usually not completely accurate. In this sense, when Zhang et al. (2018) projected their model for China onto a world map, they found that there are only median and marginal climatic suitable habitats instead of highly suitable climatic habitats in the North American region, which faces a serious problem of invasion of *E. angustifolia* (Reynolds and Cooper 2010; Collette and Pither 2015a, b a, b). In parallel, our study area was not a climatically suitable habitat either according to Zhang et al. (2018), despite we have a greatly invaded area. Then, local studies are necessary to assess the adaptability of *E. angustifolia* to new climate conditions and contribute to the knowledge of the behavior of the species at the global scale.

In Argentina, *E. angustifolia* was introduced as an ornamental. In the early 1970s, the natural occurrence of this species was observed on the banks of the Negro River and currently forms monospecific strata along the river (Klich 2000). According to INBIAR (2023) and Flora Argentina (2023), the species is reported in north Patagonia and humid Pampa regions (provinces of La Pampa, Neuquén, Río Negro, Chubut and Santa Cruz). *Elaeagnus angustifolia* is an adventive tree in drylands and a naturalized tree in irrigated areas (Steibel et al. 2000) but the distribution accurate limits still need to be discovered. According to Yansen and Biganzoli (2022), *E. angustifolia* is classified as “potentially problematic in Argentina,” so it is necessary to generate useful information to prevent possible invasions of this species.

Our research aims to generate a model that allows the relationship between the availability of chill hours with the germination of *E. angustifolia* and to make a map of the probability of seed dormancy break that allows the germination of the species in Argentina. We hypothesize that *E. angustifolia* is a species capable of germinating and establishing itself in environments that satisfy their chill hours for its germination and soil water availability for its establishment requirements. We expected germination and establishment of the species to increase as the number of chill hours in the seeds and the humidity in the soil increase.

Methods

Study area

The fruit collection area of *Elaeagnus angustifolia* was located in the phytogeographic province of Monte (Cabrera 1971; Morello et al. 2012; Oyarzabal et al. 2018), in the

Departments of Adolfo Alsina and Conesa in Río Negro province, Argentina. The climate in this region is semiarid, windy, and transitional subtemperate, especially in spring and summer, with hot summers and moderate winters. Precipitation varies in an SW-NE gradient, with averages of 280–350 mm per year and maximums in autumn and spring, presenting a high variability between years (Godagnone and Bran 2009).

Seed collection and chill hours accumulation

Eight *Elaeagnus angustifolia* forests were randomly selected along 165 km on the riverbank of Negro River between the cities of General Conesa (40.07°S, 64.46°W) and Viedma (40.8°S, 62.99°W) in the province of Río Negro, Argentina. The environmental conditions are homogeneous in this distribution range.

Ten individuals were randomly selected in each forest to collect fruits since the germination power of *E. angustifolia* presents variability among individuals (Kiseleva and Chindyaeva 2011). The fruits were collected in autumn when their harvest is recommended (Olson 1974; Hamilton and Carpenter 1975; Hybner and Espeland 2014).

A total of 5600 fruits were collected and processed as a single unit without discriminating their origin since the environmental conditions are homogeneous. The exocarp and mesocarp were removed from each fruit to obtain only the seed. The number of seeds was distributed in seven groups of 800 seeds each. Each group of seeds was stored in paper bags in a dry dark place. We considered the available chill hours (CH) in Argentina and the recommended CH range for germination of *E. angustifolia* (Heit 1967; Lindquist and Cram 1967; Olson 1974; Guilbault et al. 2012). Starting in mid-May (autumn in Argentina), every 15 days (360 h), a group of seeds was placed inside a bag filled with moist sand at a temperature between 2 and 7 °C inside a cooler without ventilation (Olson and Barbour 2008), until there were six bags inside the cooler. One group of seeds was not exposed to CH accumulation. Thus, a staggered CH accumulation time was achieved, obtaining seven levels: control (0), 360, 720, 1080, 1440, 1800, and 2160 CH.

Germination and survival

One month before the beginning of spring (August), seeds from each level of CH accumulation were sown in 20 trays (50 cm length, 34 cm width, 12 cm depth) with two different soil moisture conditions. This factor was called “Moisture” and had two groups: “half field capacity” (HFC) with 19% moisture and “field capacity” (FC) with 38% moisture (Hybner and Espeland 2014). Two soil moisture conditions were used with the intention of approaching the moisture conditions of drylands (using HFC) and river banks and irrigated

areas (using FC). Soil moisture content was kept by the gravimetric method. We drilled a series of 1 cm holes in the bottom of each tray to allow water exchange. The soil used in our experiments is clay loam which comes from areas where the species is present (Olson and Barbour 2008).

The soil was sieved with a 2 mm mesh to avoid the presence of *E. angustifolia* propagules from the seed bank. Ten trays were conditioned for the HFC moisture factor and ten trays for the FC factor. We randomly subdivided each tray into seven sectors, so each sector contained 40 seeds of each of the seven chill hours accumulation levels, obtaining ten replicates for each treatment (Moisture x CH).

Seeds were placed at a depth of 1.5–2.5 cm and germinated in a greenhouse with a photoperiod of 16 h (Bertrand and Lalonde 1985; Olson and Barbour 2008; Hybner and Espeland 2014) and a mean temperature of 17.5 °C. Seed germination and survival of seedlings in each treatment were recorded weekly for a period of 120 (Hybner and Espeland 2014) and 240 days, respectively. Survival was recorded by identifying each emerged seedling with a plastic marker.

Statistical analysis

The germination predictor model was obtained by analysis of the data with the R software (R Core Team 2022) using a Generalized Linear Model (GLM) of beta distribution, where the linear predictors were obtained on a logit scale. Then a link function was applied, and the germination probability prediction equations were established for each soil moisture condition according to the CH accumulation.

Germination probability map

We mapped the seed germination probability of *Elaeagnus angustifolia* in Argentina using the historical data of CH from 1961 to 2010. We obtained these data from the Instituto Nacional de Tecnología Agropecuaria (INTA) database, which registers regional weather information periodically from 105 monitoring stations distributed along the country. The chill hours were calculated with the Parton and Logan (1981) method. The inverse distance weighted method (IDW) was used to interpolate the spatial distribution of average chill hours. This method uses a linear-weighted combination set of sample points to determine cell values. These interpolated chill hours were used to calculate the germination probability for each raster cell, following the model formula obtained in this study:

$$P(\text{Germination}) \sim \text{Moisture} * \text{Chill hours}$$

We created two contour maps (one for each Moisture) from probabilities of germination calculated, setting an upper threshold corresponding to the maximum value of

probability obtained in the germination experiments. Analyses were performed with the statistical software R (R Core Team 2022).

Results

Germination

Elaeagnus angustifolia germination varies according to chill hours accumulation and Moisture. As predicted by the model, when seeds grow under field capacity (FC) conditions, there is a slightly negative relationship between chill hours accumulation and germination (p -value < 0.005), with a maximum predicted germination value of 29.17% corresponding to zero chill hours accumulation. However, when seeds grow under half-field capacity (HFC) conditions, the relationship between chill hours accumulation and germination becomes positive (p -value < 0.001), with the maximum germination value being 90% (Fig. 1).

When there is no chill hours accumulation, there are no statistically significant differences in germination percentages between FC (29.17%) and HFC (32.94%) ($p = 0.2926$).

These raw data of germination percentage range from 15 to 47% for FC and from 7 to 53% for HFC.

We have not observed any signs of damage in not germinated seeds.

Survival

Seedling survival was 100% for all treatments during the 240 days of monitoring, so no statistical comparisons were made.

Germination probability map

If chill hours accumulation is considered the main conditioning factor for breaking seed dormancy that allows germination, *Elaeagnus angustifolia* can germinate throughout the Argentine territory under the two soil moisture conditions studied (Fig. 2).

The germination probability of *E. angustifolia* in our country is conditioned by the soil moisture condition, based on the historical record of chill hours.

The germination probability increases towards the north of Argentina in FC soil moisture conditions (Fig. 2A). However, the germination probability rises towards the south

Fig. 1 Effect of chill hours accumulation and soil moisture condition (Moisture) on germination of *Elaeagnus angustifolia*. *CH* chill hours accumulation, *FC* field capacity condition, *HFC* half-field capacity condition, dots and triangles indicate the dispersion of the data for FC and HFC, respectively; the dotted line and the solid line indicate the model prediction for FC and HFC, respectively; the shaded area indicates the 95% CI for each prediction

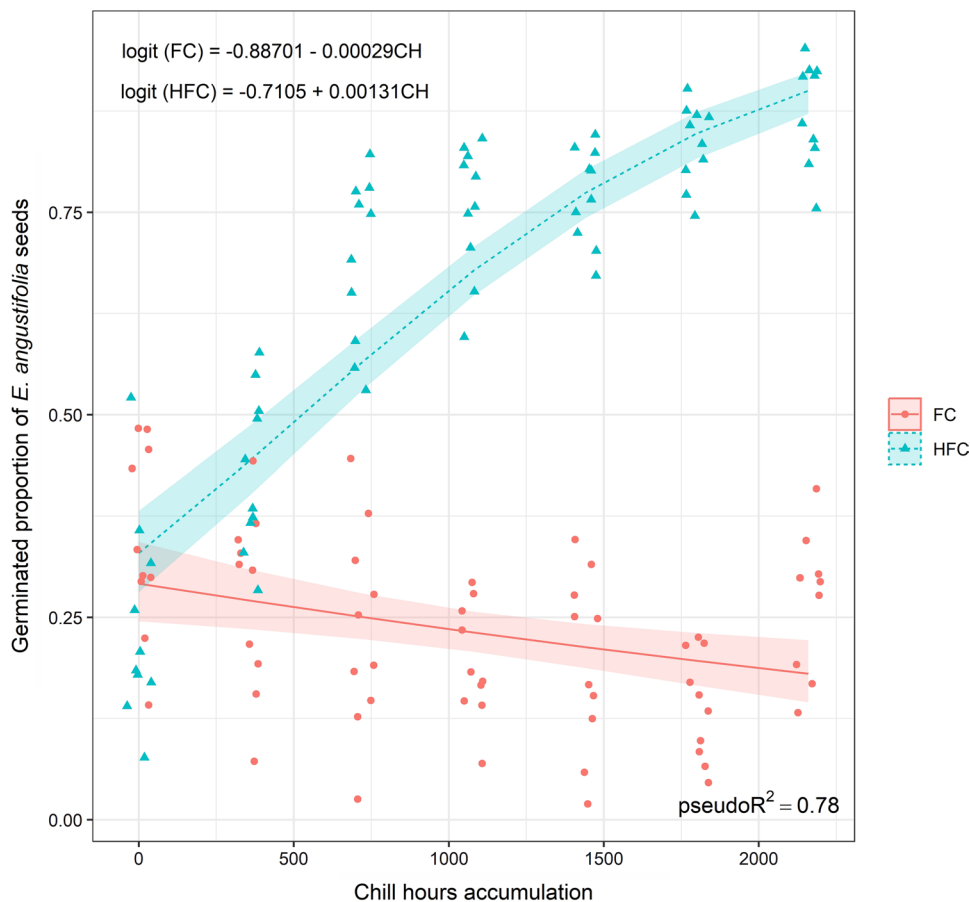
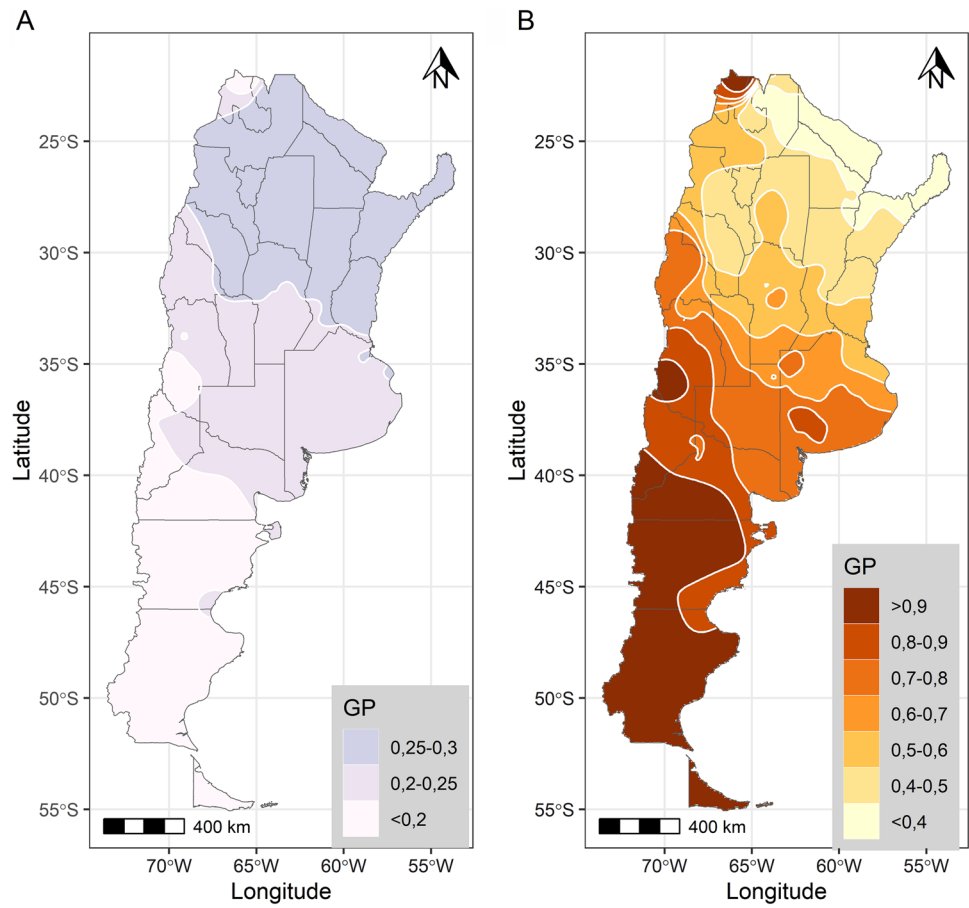


Fig. 2 Heat map of germination probability of *Elaeagnus angustifolia* related to seed dormancy breaks according to chill hours availability under the two tested soil moisture conditions. **A** under field capacity (FC) condition; **B** under half-field capacity (HFC) condition. *GP* germination probability



sector in HFC soil moisture conditions, corresponding to Argentinian Patagonia (Fig. 2B).

Discussion

The germination probability of *Elaeagnus angustifolia* increased as the CH accumulation increased only in half-field capacity treatment (HFC). Therefore, we partially reject the proposed hypothesis since, at field capacity (FC), the germination probability decreases as the CH accumulation time increases. Our germination results obtained under HFC agree with those reported by Guilbault et al. (2012), who found a positive relationship between chill hours accumulation and germination percentages in *E. angustifolia*.

It is striking that the maximum percentage of germination recorded in FC is lower than in HFC (47% vs. 90%, respectively) because *E. angustifolia* implants in uplands but prefers humid zones (Katz and Shafroth 2003; Jarnevich and Reynolds 2011; Guilbault et al. 2012). According to our hypothesis, the negative relationship (p value < 0.005) between chill hours and germination in soil with field capacity was unexpected. The lower percentage of germination found at FC than at HFC could be linked to

soil sealing due to possible overwatering. This soil structure degradation could reduce the emergence of seedlings (Sumner and Stewart 1992; Needham et al. 2004). Even so, the soil sealing effect does not explain the negative relationship between chill hours and germination in field capacity soil since, in both moisture conditions tested in our work, the soil used can be considered homogeneous. Another explanation could be that seeds spent time under water after irrigation in FC conditions, which may have caused loss of viability due to anoxia. We cannot support this idea because we have not noticed any damage in not germinated seeds, and there is evidence that *E. angustifolia* benefits from ample water availability (Katz and Shafroth 2003) and its presence is highly spatially associated with flood areas (Moody and Schook 2023; West et al. 2020). So, additional research must be carried out to clarify this behavior under different soil moisture conditions.

The maximum germination percentages recorded in our experiment were 47% in FC and 90% in HFC, while other studies obtained maximums of 23.3% (Guilbault et al. 2012), 60% (Iriondo et al. 1995), and 86% (Kiseleva and Chindyaeva 2011). The high germination percentages found and the extraordinary capacity of seed production

alert about the invasive potential of *E. angustifolia* (Rejmanek and Richardson 1996).

Our results show that when seeds are not exposed to chill hours, the germination percentages range from 15 to 47% and 7 to 53% for FC and HFC, respectively. These data differ from those provided by Kiseleva and Chindyaeva (2011), who obtained a maximum of 8.3% germination without CH accumulation. In contrast, Hogue and LaCroix (1970) showed that between 50 and 60% of seeds germinated without CH accumulation. Guilbault et al. (2012) reported that, under the same germination conditions, seeds from trees close to the distribution limit (imposed by the insufficient availability of CH) had a higher percentage of germination than those seeds from trees that were further to the limit. Cavieres and Arroyo (2000) reported similar behavior for *Phacelia secunda* J.F. Gmel. They suggest that different populations located in an altitudinal gradient have less CH accumulation requirements the less cold their habitat is. However, according to our model prediction, *E. angustifolia* needs low CH accumulation to germinate in central and northern Argentina, even though the region of origin of the germplasm used in our experiment has a high availability of CH. This seed germination success without cold stratification could be due to the genetic variability that *E. angustifolia* presents (Asadiar et al. 2012; Khadivi 2018; Khadivi et al. 2020). Rapid and efficient acclimatization due to its phenotypic plasticity and functional flexibility (Castro-Díez et al. 2004) is one of the characteristics repeated in invasive species. If exotic species rapidly adapt (Henery et al. 2010) to new biotic and abiotic conditions, then they might outcompete native species (Catford et al. 2011) and persist under future climate scenarios (Dukes and Mooney 1999; Muth and Pigliucci 2007; Williams and Jackson 2007).

The insufficient availability or even the lack of chill hours is not a limiting factor for *E. angustifolia* seed dormancy break that allows germination. Therefore, the species has the potential to germinate throughout the country, where the greater germination probability corresponds to the greater CH availability in soil conditions of HFC, which coincides with the Argentine Patagonia sector (Fig. 2B). Yansen and Biganzoli (2022) point out the presence of *E. angustifolia* in the area they call “Monte Patagonia,” which is consistent with an area of a high germination probability generated by our model. Based on their bibliographic background, these authors classify *E. angustifolia* as “potentially problematic in Argentina.” However, the lack of perception of the negative impacts (Pyšek and Jarošík 2005) or the absence of studies on the economic impact of a species could lead to an incorrect classification of *E. angustifolia*. In the USA, where the species was introduced a century earlier than in Argentina (Olson and Knopf 1986; Klich 2000; Katz and Shafroth 2003), its negative effects are widely known (Shafroth et al. 1995, 2010; Katz and Shafroth 2003; Friedman et al. 2005;

Reynolds and Cooper 2011; Mineau et al. 2011). So, the consequences of *E. angustifolia* invasion in our country could still be ignored due to the shorter time since its introduction. Therefore, further studies should be carried out to know its real danger.

The data obtained in our study warn us about the successful establishment of *E. angustifolia* after a 240-day record, where seedling survival was 100% for all treatments. This result differs from those obtained by Shafroth et al. (1995), who found that the survival of *E. angustifolia* was in a range of 1–44% depending on the depth of the subsoil water, without significant differences between two contrasting light levels (full sun and 89% shade). Several studies agree that *E. angustifolia* germinates in shade conditions (Shafroth et al. 1995; Reynolds and Cooper 2011) or even under complete canopies (Katz and Shafroth 2003), although these studies have not evaluated the survival of the seedlings. Based on our survival results and the background described above, *E. angustifolia* seedlings could achieve high survival under shade in similar environmental conditions to our study area.

In contrast to Guilbault et al. (2012), the seeds without chill hours accumulation evaluated in our research achieve a successful seed dormancy break that allows germination, so we cannot establish a distribution limit by chill hours for *E. angustifolia* in our country. According to our model, southern Patagonia seems to be the ideal place for the species, but it is actually only located in northern Patagonia. This situation could be attributable to a physical or time limitation of propagule arrival. Zhang et al. (2018) found that the relationship between the species probability presence and the annual mean temperature (AMT) has a response peak at 7.9 °C. This AMT value is close to that of Río Gallegos City (51,6226° S; 69,2181° W); therefore, we can infer that southern Patagonia is a suitable area for the germination and establishment of *E. angustifolia*.

Globally, the geographic range expansion of species distribution is an increasingly frequent phenomenon (Lenoir and Svenning 2015), which will be amplified in the future by rapid environmental change (Pecl et al. 2017). The success of invasive species is related to the coupling of their attributes with the environmental conditions of the place (Ríos and Vargas 2003; Theoharides and Dukes 2007). Therefore, if we consider the biological characteristics of *E. angustifolia*, this species is a latent risk for any environment that suffers anthropogenic or natural disturbances (Dubovyk et al. 2014). Although the behavior of *E. angustifolia* in terms of germination capacity, establishment, competitive capacity, and seed production has not been studied in humid and warm areas, at least, its possibility of germinating there should not be rejected.

The establishment and maturity of only one individual of *E. angustifolia* could lead to rapid dispersal in an uninvaded area due to the high number of fruits the species can

produce, and the high percentages of germination and survival obtained in our research. Some authors hypothesize that the success of biological invasions is determined mainly by propagule pressure rather than by the intrinsic characteristics of the species (Lockwood et al. 2005; Seebens et al. 2017; Gallardo and Vilà 2019). Management of exotic species requires releases to be kept to low levels to prevent establishment (Cassey et al. 2018). Even so, the only way to eliminate the risk of establishment is through total exclusion (Forsyth and Duncan 2001) because a small number of propagules (< 10) can lead to high establishment probabilities (Moulton et al. 2012). In addition to the dispersal risks supposed by direct human activity (e.g., implantation for ornamental use, forage use), the establishment and dispersal processes of *E. angustifolia* could be facilitated by the alteration of river flows and reservoirs, or by water extraction for irrigation (Howe and Knopf 1991; Lesica and Miles 1999; Katz and Shafroth 2003), or by frugivory birds (Abdalla 2019). We have to consider that the seedling stage is the most sensitive stage of the plant life cycle, where the emerging seedling faces a set of hazards like lack of light, water, or nutrients, the presence of predators and pathogens (Fenner and Thompson 2005). Consequently, a low percentage of germination might hinder *E. angustifolia* establishment, while it could be increased in areas with high seed dormancy breaks allowing high percentages of germination.

Associated costs to biological invasions must be considered in invasion prevention efforts to effectively use resources in developing countries (Duboscq-Carra et al. 2021), particularly in agricultural areas (Paini et al. 2016). Although in our work, we have not made estimates on the economic impacts of *E. angustifolia*, in a first analysis, Duboscq-Carra et al. (2021) found that most of the costs recorded in Argentina are associated with the invasion of *Tamarix* spp., with negative ecological, social, and economic impacts, because it invades productive lands and subsistence farming areas (Natale et al. 2008, 2012; Zilio 2019). *Elaeagnus angustifolia* invades similar environments to those invaded by *Tamarix* spp. (Dubovyk et al. 2014); therefore, *E. angustifolia* could represent an economic risk in agricultural areas. In this sense, this species is cited in the Argentinian official list of invasive alien species included in the government's National Strategy which aims to study, control, and eradicate invasive species and to improve institutional capacities to manage biological invasions (MAyDS 2022). As an integral part of this strategy, the Argentine government seeks to promote the generation of public policies to minimize the impact of biological invasions on the national economy. Despite this, *E. angustifolia* is included in Category 2 (organisms of importance for production) and we think that is necessary to reevaluate his classification in Category 1 (restricted-use species, that is, species with high environmental and socioeconomic impact, and limited

or null productive use). Identifying invasions before they occur is the most efficient strategy to prevent the introduction of invasive alien species (Fournier et al. 2019) and changing the classification of *E. angustifolia* allows a major control over the introduction and expansion of this species in areas without presence. We have cited several investigations in which the aggressiveness and speed of invasion of *E. angustifolia* are evident. So, the capacity to break seed dormancy throughout the country could lead to the germination of the species and its subsequent establishment and invasion. We think that, supported by our results and cited bibliography, the species is absent in southern Patagonia due to a propagule limitation. In this sense, we recommend minimizing the dispersal risks of *E. angustifolia* by avoiding its implantation for ornamental, forage, or restoration use across the country by the mentioned biotic characteristics in this manuscript.

In addition to contributing to the prevention of *E. angustifolia* invasion in Argentina, the information generated in our research can be helpful in other regions of the world since the significant genetic variability of *E. angustifolia* can make its environmental range of distribution unpredictable.

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Author contributions All authors designed the study. JMSL performed the experiments and statistical analyses. JMSL wrote the first draft of the manuscript, which was substantially improved through the contribution of C.P., S.T.R., and G.P.

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Data availability The raw data of experiments and R scripts are available in https://drive.google.com/drive/folders/1PypCAPpiCCJM6nX3-yqiqDODINva1c3_?usp=drive_link.

Code availability The data analysis for this paper was generated using R Studio, software version 4.2.0 (R Development Core Team 2022).

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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