Factors affecting seed germination of *Eragrostis plana* populations

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Abstract: Background: *Eragrostis plana*, introduced from South Africa, is the most important invasive perennial weed in the grasslands of Southern Brazil ("Pampa biome"), becoming a threat in neighboring countries. How temperature, light, and water potential affect seed germination is poorly understood but essential to understand its invasiveness and dissemination. Objective: This study characterized the seed germination of *E. plana* from locations in the Brazilian Biome Pampa under different conditions. Methods: Seed germination was evaluated by using a time-to-event model across a wide range of temperatures (15 to 45°C), light (total darkness or 12-h light), and water potentials (0, -0.08, -0.2, -0.25, -0.5, -0.75, -1, and -1.5 MPa). Results: *E. plana* seeds did not germinate below 15°C and at water potential lower than -0.75 MPa. Among populations, the highest germination was at 40°C (> 90%). The base temperature for germination ranged from 12.3 to 15.6°C across populations. *E. plana* did not germinate more than 40% at 25°C, except for one population (Caçapava do Sul), with a germination capacity of 73%. Light is a requirement for a fraction of the seeds as darkness inhibits the germination of a fraction of the seeds as well. Conclusions: *E. plana* could adapt to cooler temperatures, as shown by the ability of the Caçapava do Sul population to germinate at low temperatures. This ability for seed germination help to explain why *E. plana* has successfully invaded the rangelands of Southern Brazil, spreading around neighboring countries. Management strategies have to be urgently adopted to avoid its dissemination abroad.

1. Introduction

*Eragrostis plana* Ness is a threat to the Pampa biome and livestock production. With low forage quality when compared with native species, the high amount of fibrous in leaves causes damage to the livestock, reducing animal weight gains (Perez, 2015). The *Eragrostis* genus has approximately 350 grass species in tropical and subtropical regions abroad (Clayton, Renvoize, 1986). *Eragrostis plana* native to South Africa, was introduced in the 1950s to Southern Brazil (Kissmann, 1991) as a contaminant in imported seeds of *Chloris gayana* Kunth and *Eragrostis curvula* Schradter (Kissmann, 1991; Medeiros et al., 2004; Ferreira et al., 2008); this species has become a significant problem, especially in the Pampa biome grasslands. The Pampa biome has territories in Brazil, and also extends to Argentina, and Uruguay being considered one of the most important ecosystems in the world (Instituto Brasileiro de Florestas, 2022).

The Brazilian region of the Pampa biome is limited to the Rio Grande do Sul (RS) state, occupying 63% of the state; weather is subtropical, with temperatures around 18°C, formed by hills where livestock production is practiced and floodplains with low and humid areas. In the Brazilian Pampa, livestock production has been practiced since the country’s colonization; therefore, the *E. plana* invasion threat to livestock production and the biome. In 2009, it was estimated 1,000,000 ha of the Brazilian biome was invaded by *E. plana* (Medeiros et al., 2009). Neighboring countries that share the same biome, such as Uruguay, have also reported concerns about the invasion of its natural fields by *E. plana* (Boggiano et al., 2004).

Previous studies have reported difficulties controlling *E. plana*, which favors its dissemination (Goulart et al., 2009; Bastiani et al., 2021; Faleiro et al., 2022). Livestock does not graze on *E. plana* because of its high fiber and low protein content unless the plants are at the seedling or blooming stages; the inflorescence is attractive to cattle as it is soft. Thus, selective grazing of inflorescences greatly aided the proliferation and dominance of *E. plana* across the landscape, contributing to the endozoochoric seed dispersion (Lisboa et al., 2009; Schaedler et al., 2021).

Understanding seed germination requirement concerning temperature is crucial to elucidate the weed emergence pattern throughout the seasons. Temperature and light are the main factors that promote germination in soils with good water
availability (Andrade, 1995). The study of seed dispersal and local adaptations to variable climatic conditions in invasive plants are clue elements in understanding their success, propagation, and populational growth (Clements, Jones, 2021). To *E. plana*, high seed germination rates were reported at 35°C (Bittencourt et al., 2017; Maldaner et al., 2019). However, the temperature effects, water availability, and light on seed germination among populational have not been reported to date. When comparing the germination ability of two *Parthenium hysterophorus* biotypes from Australia, it was possible to identify superiority from one which might be related to its invasive potential (Bajwa et al., 2018). Thus, it was our concern to understand if there are differences in temperature, light, and water potential requirements for seed germination of *E. plana* among different populations spread around the RS state where the biome is located. Barbosa et al. (2013) using a bioclimatic model, predicted the current distribution of *E. plana* in South America using data from native and invaded regions. Also, through multivariate analysis confirmed the hypothesis of a bioclimatic niche shift during the invasion which may be a consequence of absence of enemies and competitors from its native range and/or a rapid evolutionary change after introduction.

This research aimed to evaluate and compare seed germination of *E. plana* populations across a range of temperatures, light, and water potentials. This knowledge can help to determine *E. plana*’s potential to expand to other country regions, including new areas worldwide.

### 2. Material and Methods

#### 2.1 Seed source

*Eragrostis plana* seeds were harvested from fifteen panicles from plants located in ten different counties of the RS state, Brazil, according to Table 1 and Figure 1, and stored at 5°C at least seven days to break dormancy (Medeiros et al., 2014). *E. plana* seed populations were planted in 8-L pots filled with field soil (Red-Yellow Argisol with sandy-loam texture) and kept in a greenhouse at the Federal University of Pelotas (UFPel), Capão do Leão, RS, Brazil being used to raise seed-bearing plants under identical conditions, according to Bajwa et al. (2018). The seeds were hand harvested when they have reached physiological maturity and kept under laboratory conditions (25°C) first. After one week, seeds were kept at 5°C until the beginning of the studies. Once the seeds used in the studies came from plants grown and stored under identical conditions, the effect of the maternal environment was kept to a minimum and any germination differences seen in subsequent experiments can be attributed to differences in populations. All populations received the ID: PAL, VAC, ITA, SM, CAÇ, VC, BAG, PEL, and SVP, according to Table 1.

#### 2.2 Factors affecting germination

Forty seeds per population were placed on a double layer of filter paper in Petri dishes (diameter, 9 cm), moistened with 2.5 ml of distilled water. The experimental units (Petri dishes) were arranged in a completely randomized design, with three replications. The experiments were conducted twice.

##### 2.2.1 Temperature

The seed germination was assessed under eight constant temperatures (15; 20; 25; 30; 35; 40; 43, and 45°C).

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**Table 1** - Georeferencing of the sampled locations, monthly temperature, and rainfall in each region. South latitude (S) and West (W) longitude

<table>
<thead>
<tr>
<th>ID</th>
<th>Population</th>
<th>Region</th>
<th>Location</th>
<th>Rainfall* (mm)</th>
<th>Temperature* (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL</td>
<td>Palmitinho</td>
<td></td>
<td>27°19’11” S 53°36’04” W</td>
<td>157</td>
<td>21,5</td>
</tr>
<tr>
<td>PF</td>
<td>Passo Fundo</td>
<td></td>
<td>28°13’44” S 52°28’59” W</td>
<td>159,66</td>
<td>18,69</td>
</tr>
<tr>
<td>VAC</td>
<td>Vacaria</td>
<td></td>
<td>28°27’24” S 50°59’18” W</td>
<td>146,01</td>
<td>16,08</td>
</tr>
<tr>
<td>ITA</td>
<td>Itaqui</td>
<td></td>
<td>29°10’54” S 56°28’39” W</td>
<td>116,42</td>
<td>19,96</td>
</tr>
<tr>
<td>SM</td>
<td>Santa María</td>
<td></td>
<td>29°49’51” S 53°46’33” W</td>
<td>141,82</td>
<td>20,3</td>
</tr>
<tr>
<td>CAÇ</td>
<td>Caçapava</td>
<td></td>
<td>30°32’04” S 53°26’16” W</td>
<td>128,17</td>
<td>17,5</td>
</tr>
<tr>
<td>VC</td>
<td>Vera Cruz</td>
<td></td>
<td>29°43’46” S 52°28’25” W</td>
<td>110,25</td>
<td>19,7</td>
</tr>
<tr>
<td>BAG</td>
<td>Bagé</td>
<td></td>
<td>31°11’25” S 54°19’33” W</td>
<td>128,01</td>
<td>18,66</td>
</tr>
<tr>
<td>SVP</td>
<td>Santa Vitória do Palmar</td>
<td>31°42’50” S 52°25’29” W</td>
<td>115,79</td>
<td>18,88</td>
<td></td>
</tr>
</tbody>
</table>

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**Figure 1** - Locations of populations sampling of *Eragrostis plana*, in the Brazilian (inset) and the state Rio Grande do Sul map
at 12h light based on Bittencourt et al. (2017), in growth chambers. Germinated seeds were counted and removed every 12h during 21 days. Radicle emergence (> 2 mm) was used to indicate seed germination. Germination was expressed as the proportion of seeds germinated in each Petri dish. Petri dishes were sealed with plastic film to prevent water evaporation.

2.2.2 Light

To evaluate the light effect on germination, seeds were placed in growth chambers at 40°C, under complete darkness or 12h light; this temperature was chosen based on the temperature experiment described in 2.2.1. For darkness incubation, dishes were wrapped in a double layer of aluminum foil. The germination seed count and removal occurred every 24h, for 14 days. For the darkness experiment, the germination count was performed in a dark room using a lamp with dark green light.

2.2.3 Water potential

A preliminary experiment was performed with 13 water potential levels (from 0 to -1.5 MPa), four temperature treatments (from 25 to 40°C), and light (data not shown). Based on that, eight water potential treatments: 0 (distilled water), -0.08, -0.2, -0.25, -0.5, -0.75, -1, and -1.5 MPa, at 40°C, were selected. For the water potential treatments, seeds were germinated using different concentrations of polyethylene glycol (PEG 6000) with distilled water (Michel and Kaufmann, 1973). The experimental units were arranged in a completely randomized design with four replications. Germinated seeds were counted as described in the procedure in section 2.2.1.

2.3 Statistical analysis

Data from experiments replicated in time were pooled (after they were statistically checked for differences between them (t test)). Temperature, light, and water potential experiments were analyzed using a time-to-event model. Three-parameter log-logistic model (Equation 1) fit the cumulative seed germination data (Ritz et al., 2015):

\[
F(t) = \frac{d}{1 + \exp[b \cdot \log(t) - \log(t_{50})]} = \frac{d}{1 + (\frac{t}{t_50})^b}
\]  

[1]

By definition, F is 0 at time 0. The model assumes that F(t) may approach a fraction or proportion d, between 0 and 1, as time elapses (t—→) (Ritz et al., 2015). Therefore, F is the cumulative seed germination at time t (equal to thermal time [degree-day, Cd] or days); d is the upper limit representing the germination capacity (%) of the total number of seeds; T_{50} (or WP_{50}) is the thermal time measured as thermal time (Ch); time (hours); water potential (−MPa). The 50% cumulative germination, with or without light, is represented by (d) at time T_{50} (WP_{50}); b is the slope, denoting the germination rate (Adapted from Scherner et al., 2017).

Base and maximal temperatures were estimated based on the intersection of each regression line with the abscissa when germination rates (1/T_{50}) are regressed versus temperatures. The optimal temperature (T_o) was estimated based on the intersection of these two regression lines (Dumur et al., 1990) using the intercepts and slopes of these two regression equations: T_o = (a2 - a1) / (b1 - b2).

Base water potential (Ψ_b) was estimated using the same procedure, but by regressing the germination rate (1/T_{50}) versus the water potential. The intercept of the regression line on the X-axis was used as an estimate for the base temperature and base water potential for germination (T_b or Ψ_b) (Patané et al., 2009; Patané and Tringali, 2011).

Statistical confidence intervals (95%) were estimated using the bootstrap method (Efron, Tibshirani, 1993), which estimates the base (T_b or Ψ_b); T_o and Ψ_o were used to determine the 95% confidence interval. Statistical analyses were conducted using the statistical R software in the add-on drc package (Ritz, Streibig, 2005). For all experiments, the post hoc t-test was used to test whether the parameters differed among temperature, light, and water potential (Ritz, Streibig, 2005). All the experiments were repeated twice.

3. Results and Discussion

3.1 Temperature

The optimal temperature (T_o) for E. plana germination ranged from 38.9°C to 40°C (Table 2); base temperature (T_b) fluctuated from 12.3°C for SM to 15.6°C for PF, VC, and PAL populations, being significantly variable (Table 2). The time-to-event model estimated the Tm from 45.1 to 46.0°C. The germination capacity (d), on average, was 47.2% when the temperature was above 40°C (Table 2). However, when the temperature was below 35°C, the germination capacity was less than 80%; the exception was the CAÇ population, which estimates the base (Tb or Ψ_b) (Patané et al., 2009; Patané and Tringali, 2011).

When germination was minimal or inexistent (i.e., 15, 20, or 45°C), the parameters could not be estimated. Again, the exception was the CAÇ population with 57% of germination capacity at 20°C (data not shown).

3.2 Light

Germination capacity for each population was over 90% with 12h-light and 56% in the dark (Figure 3, Table 4 in supplementary file). The time to reach 50% germination (T50) and the slope around T50 did not differ among populations under 12h-light. In the dark, the SVP population needed 19h longer than the same population grown in the light condition, to attain 50% of germination. The VC population germinated 76% in the dark, whereas the PEL and PF populations germinated only 33 and 34%, respectively (Table 4, in supplementary file).

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Therefore, there is photoblastic variation in seed germination among *E. plana* populations from Southern Brazil.

### 3.3 Water potential

Decreasing water potential reduced the germination capacity of *E. plana* populations. The highest seed germination (93.5%) was achieved at 0 MPa (Figure 4, Table 5 in supplementary file). Decreasing water potential from 0 to -0.25 MPa caused a 38% reduction in germination capacity. For BAG, PEL, PF, CAÇ, and SVP populations, germination ceased at -0.5 MPa, while the others could still germinate at -0.75 MPa. No germination was observed when the water potential was -1 MPa or lower. All populations showed a delay in germination under water potentials ≤-0.25 MPa based on comparisons of T50 values. The SVP population was sensitive to dryer germination conditions when compared with others, showing a significant reduction in germination capacity (74%) at -0.08 MPa compared to 93% at 0 MPa (Figure 4, Table 5 in supplementary file).

The *E. plana* populations showed a broad range of germination temperatures. Although the majority germinated better between 35 and 40°C, others achieved germination at lower temperatures (CAÇ population, 20°C – data not shown) and even in high temperatures; the PF population needed a high temperature (40°C) to achieve at least 83% of germination. This result is unusual for *E. plana* (Kissman, 1991), which give *E. plana* seedlings advantages to compete with summer species from the Pampa Biome, for the case of CAÇ when the weed seeds will germinate first; or to survive in high-temperature regions where many species would struggle to survive (i.e., for the PF). Having a germination amplitude favors the invasive plant to the detriment especially of the native plants of the biome.

In the climate of Southern Brazil, including the RS state, the main area invaded by *E. plana* is subtropical temperate (Instituto Brasileiro de Geografia e Estatística, 2004). The average precipitation varies between 1,299 mm and 1,800 mm, with higher rainfall recorded in the north and northeast of the state (Figure 1). The North region, where the PF population was from, usually shows higher temperatures, especially during the spring season when *E. plana* germinates. The knowledge of base temperature (Tb) can help establish weed management strategies, mainly in places where winter temperature is low and rises in the spring, enabling the prediction of time to germination initiation (Guillemin et al., 2013). The estimated Tb values in the present study (12.3 to 15.6°C) were higher than that recorded for *E. plana* seeds, 10.7°C, collected from Abelardo Luz County (Santa Catarina State), where the mean annual temperature is 17.3°C (Bittencourt et al., 2017).

Differential germination response to temperature reflects a localized adaptation of the populations (Guillemin et al., 2013); in other words, the evolution of ecotypes. The low base germination temperature for CAÇ compared to other *E. plana* populations from the RS state might be explained by its origin. CAÇ seeds were collected from a region with a colder mean annual temperature than the other regions represented in the study (Figure 1). CAÇ has adapted to lower temperatures, being the only one that reached >50% germination at 20°C (data not shown) and 73% at 25°C. All other populations did not germinate well under these temperatures. Even the SM population which showed Tb close to CAÇ. Similar alterations in base germination temperature have been reported previously for *Echinochloa crus-galli* from different origins. Seeds from Northwestern France had an estimated Tb of 6.2°C (Guillemin et al., 2013); seeds from Italy had a Tb of 10°C (Sartorato, Pignata, 2008) and 13°C for seeds from California, USA (Steinmaus et al., 2000).

Changes in germination behavior enable ecotypes to compete and thrive in a locality atypical of their native range (Forcella et al., 2000). For that, *Eragrostis Wolf*, the largest genus comprising more than 350 species in the subfamily Chloridoideae (Poaceae) (Van den Borre, Watson, 1994), has been found distributed in diverse climates and environments.
Figure 2 - Cumulative germination [%] of *Eragrostis plana* populations: Bagé (BAG), Palmitinho (PAL), Pelotas (PEL), Santa Maria (SM), Vera Cruz (VC), Vacaria (VAC), Itaqui (ITA), Passo Fundo (PF), Caçapava do Sul (CAÇ), Santa Vitória do Palmar (SVP) with days at five temperatures (25, 30, 35, 40 and 43 ºC), according to Ritz et al. (2013). UFPel, Capão do Leão/RS, 2017
Figure 3 - Cumulative germination (%) of *Eragrostis plana* populations: Bagé (BAG), Palmitinho (PAL), Pelotas (PEL), Santa Maria (SM), Vera Cruz (VC), Vacaria (VAC), Itaqui (ITA), Passo Fundo (PF), Caçapava do Sul (CAÇ), Santa Vitória do Palmar (SVP) in regards to days at light or darkness condition, according to Ritz et al. (2013). UFPel, Capão do Leão/RS, 2017
Figure 4 - Cumulative germination (%) of Eragrostis plana populations: Bagé (BAG), Palmitinho (PAL), Pelotas (PEL), Santa Maria (SM), Vera Cruz (VC), Vacaria (VAC), Itaqui (ITA), Passo Fundo (PF), Caçapava do Sul (CAÇ), Santa Vitória do Palmar (SVP), in relation to days, for six water potentials (-MPa) at 40°C, according to Ritz et al. (2013). UFPel, Capão do Leão/RS, 2017
ecological environments. Probably, because of their wide range and complex ploidy levels associated with the genome size variation, which affects the evolution and the answer to environmental factors (Hutang et al., 2023). The E. plana populations of RS State originated theoretically from South Africa via contaminated imported seeds. Caratti et al. (unpublished data) confirmed this using ITS4, ITS5, rps16x2F2, and trnK molecular markers, finding that interpolation genetic distance matched with the origin proposed to populations in RS.

Light is not mandatory for seed germination of several weedspecies, including many Eragrostis species. Nonetheless, light improves germination. For example, Eragrostis tenuifolia had 77% of germination under 12-h light, and 33% under complete darkness (Bittencourt et al., 2017), Eragrostis tef reached >70% germination under continuous light and just 56.5% in absolute darkness (Tiryaki, Kaplan, 2019), and Eragrostis tenella germination was inhibited, for all, in darkness condition (Chauhan et al., 2017). In general, E. plana populations germinated well with light, corroborating the findings of Bittencourt et al. (2017) and Maldaner et al. (2019). But also, some E. plana populations (PAL, VC, CAÇ) had important germination (>70%) in the darkness condition. The seed germination under darkness could contribute to the invasiveness potential (Bittencourt et al., 2017) and would explain, at least partially, the E. plana successful expansion in rangelands in Southern Brazil. The no dependence on light for seeds to germinate may favor invasion due to the advantage over native forage species, for example. Another advantage for CAÇ population, in addition to the ability to germinate at lower temperatures.

Water availability is a key factor affecting seed germination (Bradford, 2002); seeds can differ in tolerance to water deficit between species. Previous research investigating E. plana seed germination showed increased on that from 0 to 98% as water potential increased from −1.2 to 0 MPa (Bittencourt et al., 2017); the water restriction necessary for a 50% reduction of maximum germination in E. plana was estimated at approximately −0.4 MPa (Bittencourt et al., 2017). In our research, in general, germination ceased at −0.5 MPa; some populations could still germinate at -0.75 MPa. For Urochloa brizantha, a summer forage, it was observed a reduction of 50% of maximum germination at a water potential of −0.5 MPa (Garcia et al., 1998). The tolerance to germinate in low water potentials may suggest the result of adaptation to water deficit common at Pampa biome during the summer season.

As light is not a limiting factor for E. plana germination, this may facilitates the establishment of the weed across expansive, diverse rangeland. Populations that germinate better under light can be managed by cultivating a higher-density ground cover. Having a thick vegetation cover minimizes new germination and the emergence of E. plana. A good coverage of native species tends to keep soil temperature milder (lower than 39/40C), which reduces chances of E. plana seed germination. This can be done by reducing the animal load per paddock to avoid overgrazing. In other words, allow the native species to establish better and maintain the soil coverage, with a correct graze system (not overgrazing). This management strategy has been promoted but rejected by cattle ranchers who insist on keeping high-density cattle loads which favors pasture degradation. Our data support the recommendations by Brazilian Agriculture Research Corporation (Embrapa) for preventing new infestations or mitigating the expansion of existing invasions (Perez, 2015).

Our study showed that E. plana could invade habitats beyond Southern Brazil, as also indicated by Donohue et al. (2010) and also Barbosa et al. (2013). It should be a concern once the species has the potential to become an invasive plant in different areas, even where the weather use to be colder. In evolutionary terms, it is expected that E. plana in Southern Brazil and the Pampa biome region will develop germination mechanisms depending on the climatic characteristics of the propagation zone. Strategies to manage E. plana should focus on avoiding new seed production, associated with the correct pasture management. Also, ideal conditions, based on temperature and light, for seed emergence can be avoided. These are important and urgent steps to be adopted by farmers.

4. Conclusions

Seeds of E. plana populations showed an optimum temperature for germination from 38.9 to 40C. Light is not mandatory for seed germination, but populations of the invasive show higher germination capacity under this condition. In addition, E. plana populations are moderately tolerant to water stress. It is interesting to consider there is an indicative of adaptive evolution to seed germination at lower temperatures (≥ 20C), as shown by one of the populations investigated.

Author’s contributions

All authors read and agreed to the published version of the manuscript. FPL, FCC, NR, and AS: conceptualization of the manuscript and development of the methodology. FCC and MOB: data collection and curation. FCC and AS: data analysis. FCC, FPL, and NR: data interpretation. FPL, LAA, and NR: funding acquisition and resources. FPL and FCC: project administration. FPL: supervision. FPL and FCC: writing the original draft of the manuscript. FPL, FCC, NR, LAA, and AS: writing, review and editing.

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