Are the invasive grasses *Cynodon dactylon* and *Eragrostis plana* more phytotoxic than a co-occurring native?

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Abstract. Allelopathy, the release of phytotoxins by plants, may be a key mechanism by which an invasive species displaces residents. However, methodological procedures are still questioned in the literature. We evaluated the phytotoxic effects of *Cynodon dactylon* and *Eragrostis plana*, the most invasive grasses of the Río de la Plata grasslands. Although allelopathy has been claimed as one of the possible mechanisms involved in the establishment and impact of these species, no empirical evidence has convincingly supported it. We performed a germination experiment to assess the effect of both invasive species on *Eragrostis bahiensis*, a native grass from the region. We used aqueous extracts from fresh and dry leaves of three donor species: two invasive (*C. dactylon* and *E. plana*) and one co-occurring native from the introduced range (*Coelorachis selloana*). This approach allows to relativize the invasive species effect from resident species, according to the novel weapon hypothesis. As a result, only aqueous extracts from dried leaves inhibited the germination and early growth of *E. bahiensis*. The magnitude of these effects varied between donor species, but there was no consensus to conclude both invasive species had greater effects. *Cynodon dactylon* caused the strongest inhibitory effect on *E. bahiensis*. However, the native *C. selloana* also presented a potent inhibitory effect, stronger than the well-known invasive *E. plana*. Thus, the role of allelopathy on *E. plana* invasion in the Río de la Plata grasslands should be questioned. Finally, we addressed some suggestions for improving experimental design for testing the novelty of phytotoxic effects in the introduced range.

[Keywords: bermudagrass, capim Annoni, invasion, lovegrass, phytotoxins, Río de la Plata grasslands]

Resumen. ¿Las gramíneas invasoras *Cynodon dactylon* y *Eragrostis plana* son más fitotóxicas que una nativa coexistente? La alelopatía, que es la liberación de fitotoxinas por parte de las plantas, puede ser un mecanismo clave por el cual una especie invasora desplaza a las especies nativas residentes. Sin embargo, en la literatura todavía se cuestionan los procedimientos metodológicos utilizados. En este trabajo se evaluaron los efectos fitotóxicos de las gramíneas más invasoras de los pastizales del Río de la Plata: *Cynodon dactylon* y *Eragrostis plana.* Aunque se sugiere que la alelopatía es uno de los mecanismos involucrados en el establecimiento y el impacto de dichas especies, hay pocas evidencias que lo respaldan de forma convincente. Se realizó un experimento para evaluar el efecto de ambas especies invasoras sobre la germinación de *Eragrostis bahiensis*, una gramínea nativa de la región. Se utilizaron extractos acuosos de hojas frescas y secas de tres especies donantes: las dos invasoras (*C. dactylon* y *E. plana*) y una nativa (*Coelorachis selloana*). Este enfoque permite relativizar el efecto de las especies invasoras de las nativas residentes, en el marco de la hipótesis clásica de armas novedosas (*novel weapon hypothesis*). Como resultado, sólo los extractos acuosos de hojas secas inhibieron la germinación y el crecimiento temprano de *E. bahiensis*. La magnitud de estos efectos varió entre las especies donantes, sin consenso para concluir que ambas especies invasoras tendrían mayores efectos. *Cynodon dactylon* causó el mayor efecto inhibidor sobre *E. bahiensis*. Sin embargo, la especie nativa *C. selloana* también presentó una inhibición potente; incluso, fue mayor a la que presentó *E. plana*. Así, el rol de la alelopatía en la invasión de *E. plana* en los pastizales del Río de la Plata debería ser cuestionado. Por último, abordamos algunas sugerencias para mejorar el diseño experimental para evaluar cuán novedosos son los efectos fitotóxicos en la comunidad invadida.

[Palabras clave: gramilla, capín Annoni, invasión, fitotoxinas, pastizales del Río de la Plata]

INTRODUCTION

It is well known that the spread of invasive species causes significant effects worldwide by altering biodiversity and ecosystem functioning (Hejda et al. 2009; Vilà et al. 2011; Pysek et al. 2012). Although much attention has focused on describing and quantifying the invasion impact on natural ecosystems, the underlying mechanisms behind these effects have been less addressed (Levine et al. 2003). Allelopathy has been suggested as one of the most likely mechanisms by which invasive plants succeed and displace resident species from the recipient community (Inderjit et al. 1995, 2011; Callaway and Aschehoug 2000; Ridenour and Callaway 2001; Bais et al. 2003; Fitter 2003; Hierro and Callaway 2003; Callaway and Ridenour 2004; May and Baldwin 2011). This mechanism has taken particular attention in agroecosystems, where some non-native species can be considered problematic for crop production (Inderjit et al. 1995). Some invasive plants release phytotoxins that have the potential to inhibit the germination and/or growth of neighboring individuals from the recipient community, avoiding competition and thus increasing the success of invasion (May and Baldwin 2011). Therefore, invader's allelochemicals have been considered "novel weapons" in the introduced range, as they are new to the invaded recipient communities. Allelochemicals can provide competitive advantages to an invader in the introduced range because neighbors may not have co-evolved to tolerate these compounds and resist the inhibition (Callaway and Aschehoug 2000; Ridenour and Callaway 2001; Bais et al. 2003; Fitter 2003; Callaway and Ridenour 2004; Inderjit et al. 2011; May and Baldwin 2011).

In the Río de la Plata grasslands, and particularly in Southern Brazil and Uruguay, *Eragrostis plana* Nees (locally named as capín Annoni, capim Annoni or lovegrass) and *Cynodon dactylon* (L.) Pers. (gramilla or bermudagrass) are considered two of the most invasive grasses of the region (Masciadri et al. 2010; Fonseca et al. 2013; Guido et al. 2016). These invasive species have become dominant, out'compete natives or even establish in monospecific stands in some grasslands, mostly associated to productive intensification (Medeiros and Focht 2007; Bresciano et al. 2014; Guido et al. 2016; 2017; Jaurena et al. 2016; Baggio et al. 2018). However, the mechanisms by which *E. plana* and *C. dactylon* succeed and replace resident vegetation are

poorly understood. Many studies have shown phytotoxic effects of *E. plana* on plants and claimed that allelopathy could be a possible establishment strategy (Coelho 1986; Ferreira et al. 2008; Favaretto et al. 2011, 2015, 2019; Cecchin et al. 2017; Bittencourt et al. 2018a, b; Scheffer-Basso et al. 2019). However, these studies have been restricted to laboratory assays, using methods that do not mimic natural conditions. Regarding *C. dactylon*, a widespread weed, studies have shown its phytotoxicity around the word (e.g., Chou and Young 1975; Horowitz and Friedman 1971; Delachiave et al. 1999; Smith et al. 2001). Yet, most of them are related to its effects on cultivated plants and have specific aims related to productivity in non-natural ecosystems, but none of them included the Río de la Plata grasslands as a target study site.

To better comprehend the allelopathic potential of an invasive species, we think some methodological aspects should be simultaneously included in bioassays, which were almost neglected in studies about *C. dactylon* and *E. plana.* Most of the studies have used as recipient species cultivated plants that are also exotic in the introduced range, such as *Lycopersicon esculentum*, *Lactuca sativa*, *Trifolium repens*, *Lolium multiflorum* and *Setaria sphacelata* (Coelho 1986; Delachiave et al. 1999; Ferreira et al. 2008; Favaretto et al. 2011). Many of these species do not even have the potential to co-occur with *C. dactylon* and *E. plana* in natural conditions. In order to investigate the novel weapon hypothesis, we consider recipient species should be native from the introduced range, as allelopathy would represent a new but possible way of interaction between the invader and species of the resident community (Callaway and Aschehoug 2000; Callaway and Ridenour 2004). Furthermore, it is known that almost all species could be phytotoxic at some level in laboratory assays (Silva et al. 2017), thus phytotoxicity can be considered meaningful if compared among others donor species (e.g., Del Fabbro et al. 2013). Regarding invasion process, including a common native plant as a donor origin control is crucial to disentangle the relative effect of an invader in the recipient community (i.e., invasive vs. native effect; Vilà and Weiner 2004; Del Fabbro et al. 2014; Dresseno et al. 2018; Guido et al. 2019). Therefore, if the phytotoxic effect of an invasive species is greater than the effect of a native species in the introduced range, this may provide evidence that effects could be related to species origin and co-evolutionary

history, enhancing plant invasiveness. Finally, most of the studies about *E. plana* phytotoxicity obtained aqueous extracts from leaves and/or root biomass after drying in an oven and/or grounding them into a powder (Favaretto et al. 2011, 2015; Scheffer-Basso et al. 2019). These post-harvesting methods aim to maximize the extraction of bioactive substances but, are far from simulating field conditions.

In this study, we explored a mechanism that may contribute to explain the invasion success of *C. dactylon* and *E. plana*, the most invasive grasses of Uruguayan and Southern Brazil natural grasslands. We evaluated the phytotoxic effects of aqueous extracts from fresh and dry leaves of both species, as leaves have shown greater phytotoxicity than other plant organs (Favaretto et al. 2011, 2015). We tested effects on germination and leaf emergence of a recipient species that is native from the introduced range (*Eragrostis bahiensis* Roem. and Schult). To relativize invasive species effects, we also evaluated the phytotoxicity of a co-occurring and widespread native grass from the introduced range (*Coelorachis selloana* [Hack.] A. Camus). We expected that both donor invasive species, and regardless the extraction method, would cause greater inhibitory effects than the co-occurring native species, according to the novelty that an invasive species would present in its introduced range.

MATERIALS AND METHODS

Plant material

We used as donor species two invasive grasses (*C. dactylon* and *E. plana*) and one native grass from the Río de la Plata grasslands (*C. selloana*). Plants were collected in the South Eastern region of the Río de la Plata grasslands, one of the largest temperate area of natural grasslands in the world (Soriano 1992), and where all the selected species co-exist. The collection points were close to each other to guarantee biomass harvest at the same day for all donor species, since changes in abiotic conditions, such as weather status, can affect allelochemical production (e.g., Dayan 2006). On 15th July 2019, we harvested leaves from non-flowering steams of *C. dactylon* and *C. selloana* in INIA Treinta y Tres experimental station (33°15'31.11'' S - 54°24'7.76'' W, Uruguay). Since in this location the invasive *E. plana* was scarce, we had to collect its biomass 100 km away (34°2'33.24'' S - 54°46'59.80'' W,

Mariscala, Uruguay). The recipient species was *E. bahiensis,* a plant that is commonly found in the Río de la Plata grasslands, including in the region where the donor species were collected. Seeds of many individuals of *E. bahiensis* were also collected in Treinta y Tres, Uruguay. A previous germination test showed its high and fast germination (nonpublished data), which are optimal characteristics for being the recipient species in a short-term study.

Aqueous extract preparation

The method for aqueous extract preparation was modified from previous studies in the region with *E. plana* (Ferreira et al. 2008; Favaretto et al. 2011, 2017; Scheffer-Basso et al. 2019). For each donor species, we prepared two types of aqueous extracts at 15% concentration (i.e., 0.15 g/mL) that differed in the post-harvest processing: fresh and dry leaves. Thus, the experiment was the combination of two factors, post-harvesting method (two levels) and the identity of the donor species (three levels), which resulted in six different aqueous extracts (i.e., treatments): 1) *C. dactylon* fresh; 2) *C. dactylon* dry; 3) *E. plana* fresh; 4) *E. plana* dry; 5) *C. selloana* fresh, and 6) *C. selloana* dry. In addition, distilled water was used as the negative control of phytotoxicity. Before preparing the aqueous extracts, we disinfected collected leaves with chlorinated water (0.5% of sodium hypochlorite solution for 10 minutes) to avoid bacteria and fungi proliferation, and then immediately washed with abundant water. For preparing the dry extracts, part of the leaves were dried in an oven at 60 °C for 48 h. After that, 15 g of fresh or dry leaves were cut in small pieces (between 3 and 5 cm) and soaked in 100 ml of distilled water for 48 h at 20 °C under darkness (i.e., avoiding oxidative processes). Finally, the material was filtered. The pH values from the six aqueous extracts, plus the distilled water, were measured with pH test strips.

Germination experiment

Each experimental unit consisted of a Petri dish (*ca.* 100 mm in diameter) with two layers of filter paper. We placed 25 seeds of the recipient species *E. bahiensis,* forming a grid of five rows by five columns, and moistened with 4 mL of extract (or distilled water for control). Thus, we obtained 35 Petri dishes, as a result of six treatments, plus water control, with five repetitions each (*n*=5). The experiment was conducted in a growth chamber with

oscillated temperature (20 °C in darkness and 30 °C in lightness) at a 12 h-photoperiod. Petri dishes were sealed with plastic film and randomly placed in the growth chamber every day. The experiment lasted six days, according to the stabilization of the germination in the control treatment. Germinated seeds in each Petri dish were counted daily to calculate total germination percentage and germination rate index (GRI) for the six days interval as:

$$GRI(\%d) = \sum_{i=2}^{6} [(G_i - G_{i-1}) / i]$$

where i is the germination count day, varying from day 2 to day 6, G_i is the percentage of seeds germinated by day i, and G_{i-1} is the percentage of seeds germinated after the previous count day (Maguire 1962; Kader 2005). At the end of the experiment, we also recorded the percentage of seedlings with emerged leaves for each Petri dish for evaluating the difference in initial growth.

Data analysis

We evaluated the effect of each factor and its interaction (i.e., post-harvesting method; donor species; post-harvesting method x donor species) on response variables (i.e., percentage of germination, GRI and the percentage of seedlings with leaves). When the interaction of both factors was significant, we also evaluated for differences between aqueous extracts (pair-wise comparisons between the six treatments). For evaluating the phytotoxicity, we did pair-wise comparisons between each aqueous extract and control (distilled water). Aqueous extracts that did not differ from control were not considered phytotoxic. For all these comparisons, we used ANOVAs with permutation testing, which is free of assumptions about normal distribution in the data (Manly 2007).

As pH values could vary between the different aqueous extracts, and this may be an artifact for the outcoming results (e.g., see Silva et al. 2017 review), we evaluated the relationship between the pH of the six aqueous extracts plus control, and the percentage of total germination of each Petri dish (*n*=35). For that, we used a simple linear model and evaluated its significance by permutation test (Manly 2007). All the analyses were done in MULTIV software (available at http://ecoqu a.ecologia.ufrgs.br). For all permutation tests (ANOVAs and lineal model) we used 10000 permutations.

RESULTS

We observed different pH values for the aqueous extracts regarding species identity and postharvest method (see Table 1). However, there was not a relationship between total percentage of germination and pH values (data not shown; R^2=0.06; P=0.175). Both factors, post-harvesting-method and donor species, and its interaction, had a significant, or marginally significant, effect on germination (P=0.001), GRI (P=0.001) and leaf emergence (P=0.07), of the recipient species *E. bahiensis*. However, only the aqueous extracts from dried biomass of donor species showed inhibitory effects when compared to control (distilled water). Furthermore, the magnitude of these inhibitory effects varied between the identity of the donor species and none consensus was found between their origin (i.e., native or invasive) (Figures 1-3). See for statistical details of the analysis in supplemental material.

At the end of the experiment, the germination of *E. bahiensis'* seeds exposed to extracts from fresh leaves of *C. dactylon* (84.8%), *E. plana* (79.2%) and *C. selloana* (75.2%) was similar to control (80%; P>0.05 for the three cases). Yet, aqueous extract from dried leaves of *C. dactylon* showed the strongest inhibitory effect on *E. bahiensis'* germination (0%) and GRI (Figures 1-2). Furthermore, the native *C. selloana* had greater negative effect on its germination (9.6%) and GRI (1.75) than the invasive *E. plana* (53.9% and 12.7, respectively, for the percentage of germination and GRI) (Figures 1-2). Regarding the early growth of seedlings, *C. dactylon* and *C. selloana* had both similar negative effects on the percentage

Table 1. pH values for the aqueous extracts of fresh and dry leaves of *Cynodon dactylon*, *Eragrostis plana* and *Coelorachis selloana*. Control is represented by distilled water.

Tabla 1. pH de los extractos acuosos de hojas frescas y secas de *Cynodon dactylon*, *Eragrostis plana* y *Coelorachis selloana*. El tratamiento control fue representado por agua destilada.

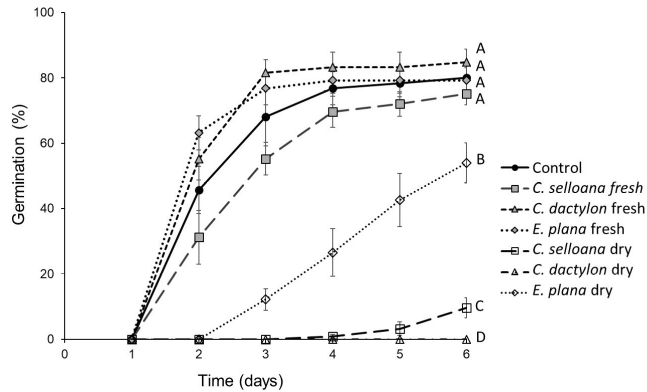| Donor species	Fresh	pH Dry
C. dacylon	5.5	6.5
E. plana	5.5	4.5
C. selloana	5	5.5
Control	6.5	

Figure 1. Cumulative percentage of germination (*n*=5) of the recipient native grass *Eragrostis bahiensis* during the six days of exposure to the aqueous extracts from fresh(grey) and dry leaves (white) of *Cynodon dactylon*, *Eragrostis plana* and *Coelorachis selloana*. Control (black) refers to distilled water. Different letters indicate significant differences (*P*<0.05) between aqueous extracts at the end of the experiment (day six).

Figura 1. Porcentaje de germinación acumulado (*n*=5) de la gramínea invasora receptora *Eragrostis bahiensis* durante los seis días de exposición a los extractos acuosos de hojas frescas (gris) y secas de (blanco) *Cynodocn dactylon*, *Eragrostis plana* y *Coelorachis selloana*. El tratamiento control (negro)se refiere a agua destilada. Letras distintas indican diferencias significativas (*P*<0.05) entre los extractos acuosos al final del experimento (sexto día).
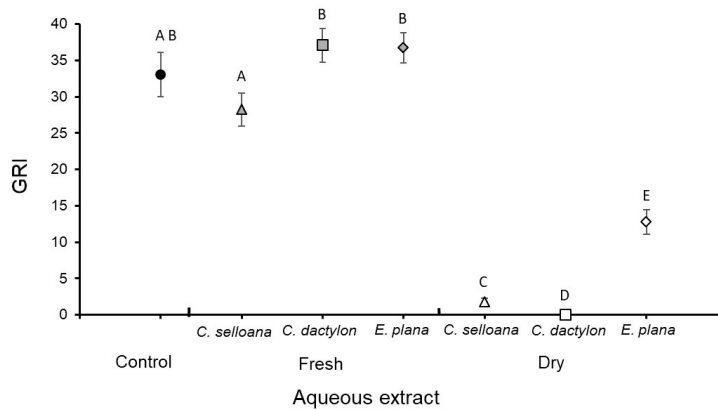

Figure 2. Germination rate index (GRI) of the recipient native grass *Eragrostis bahiensis* during the six days of exposure to the aqueous extracts from fresh (grey) and dry leaves (white) of *Coelorachis selloana*, *Cynodon dactylon* and *Eragrostis plana*. Control (black) refers to distilled water. Different letters indicate significant differences (*P*<0.05) between aqueous extracts.

Figura 2. Índice de tasa de germinación (GRI) de la gramínea receptora *Eragrostis bahiensis* durante los seis días de exposición a los extractos acuosos de hojas frescas (gris) y secas (blanco) de *Cynodon dactylon*, *Eragrostis plana* y *Coelorachis selloana*. El tratamiento control (negro) se refiere a agua destilada. Letras distintas indican diferencias significativas (*P*<0.05) entre los extractos acuosos al final del experimento (sexto día).
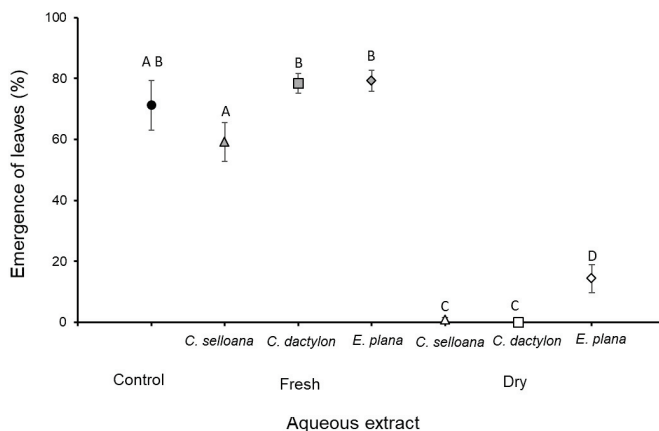

Figure 3. Percentage of *Eragrostis bahiensis* seedlings with emerged leaves (%) during the six days of the exposure to the aqueous extracts from fresh (grey) and dry leaves (white) of *Coelorachis selloana, Cynodon dactylon* and *Eragrostis plana*. Control (black) refers to distilled water. Different letters indicate significant differences (*P*<0.05) between aqueous extracts.

Figura 3. Porcentaje de plántulas de *Eragrostis bahiensis* con hojas emergidas (%) durante los seis días de exposición a los extractos acuosos de hojas frescas (gris) y secas (blancas) de *Cynodon dactylon, Eragrostis plana* y *Coelorachis selloana*. El tratamiento control (negro) se refiere a agua destilada. Letras distintas indican diferencias significativas (*P*< 0.05) entre los extractos acuosos.

of seedlings with emerged leaves (0% and 0.8%, respectively), and these effects were significantly stronger than *E. plana´*s (14.4%) (Figure 3).

Discussion

The grasses *C. dactylon* and *E. plana* are some of the most invasive species in the Río de la Plata grasslands (Masciadri et al. 2010; Fonseca et al. 2013; Guido et al. 2016). Although, allelopathy has been claimed as one of the possible mechanisms involved in the establishment of these species, we consider that the available information is not enough to make that assumption. The occurrence of allelopathy implies that 1) a species presents phytotoxins; 2) effects are observed in conditions as similar to natural as possible, including controls and recipient species that co-occur with the donor; 3) an inhibition pattern is shown in the field; 4) and the possibility that other factors explain the pattern must be ruled out (Silva et al. 2017). Current knowledge about *C. dactylon* and *E. plana* has not passed from step one, as laboratory assays have only demonstrated that these grasses affect germination and/or growth of other plants (Horowitz and Friedman 1971; Coelho 1986; Delachiave et al. 1999; Smith et al. 2001; Ferreira et al. 2008; Favaretto et al. 2011, 2015, 2019; Cecchin et al. 2017; Bittencourt et al. 2018a,b; Scheffer-Basso et al. 2019). In this study, we performed a short and simple germination experiment for going forward to the step 2) by simultaneously considering: a) a native species as recipient plant, b) a native species as donor plant to relativize the invader´s impact in the introduced range, and c) extraction procedures that attempted to approach natural conditions. As a result, we observed that fresh leaf aqueous extracts of donor species were not phytotoxic, and only aqueous extracts from dried leaves inhibited the germination and early growth of *E. bahiensis*, a native grass of the region. Moreover, the magnitude of these inhibitory effects varied between the identity of the donor species, and there was no consensus to conclude both invasive species had greater effects than the co-occurring native. These results indicate that further discussion needs to come to light about the underlying mechanisms behind the invasion successes of both species.

Regarding post-harvesting method results, only dry leaves from *C. dactylon*, *E. plana* and *C. selloana* presented an inhibitory effect

on *E. bahiensis* germination and seedling development. Previous studies that have suggested the allelopathic effect of *E. plana* have also used biomass that was subjected to a drying period and even grounded it into powder (e.g., Favaretto et. al. 2011, 2015), which may enhance compounds concentration at unreal levels. Regarding *C. dactylon*, we did not find studies focused on evaluating its phytotoxicity in the Río de la Plata grasslands. The postharvest processing can interfere in the activity of the compounds and may have little or no relation to a field situation (Inderjit and Dakshini 1995; Inderjit et al. 2005). Scheffer-Basso et al. (2019) demonstrated that aqueous extracts of *E. plana* had different phytotoxic effects depending on the phenological stage (vegetative or flowering steams) and drying process (with or without). The aqueous extract of dry leaves from non-flowering steams of *E. plana* showed a greater inhibitory effect on *L. sativa* germination and growth (Scheffer-Basso et al. 2019). Yet, the extract from fresh leaves of non-flowering steams at 15% of concentration, same as used in our experiment, did not show any phytotoxicity on *L. sativa* (Scheffer-Basso et al. 2019). Moreover, Ferreira et al. (2008) used fresh biomass of *E. plana*, which was chopped and placed in germination boxes at different levels of cover, but they did not observe a phytotoxic effect on *L. sativa´*s germination. Although laboratory assays can simplify the complex reality by controlling some important factors, many of the previous employed methods for testing *E. plana* allelopathy are difficult to extrapolate to nature, thus we question the role of allelopathy for *E. plana´*s invasion. We consider that results based on more realistic experimental designs are missing to demonstrate that allelopathy can be a mechanism related to its invasiveness.

Considering a recipient species that is native from the study system (*E. bahiensis* in our case) is important to evaluate whether the allelochemicals of the donor species present novelty effects for the recipient community. Among studies focused on *E. plana* effects, this consideration has only been implemented in the study of Ferreira et al. (2008), in which *Paspalum notatum* was included as a recipient species, a common native grass of the Río de la Plata grasslands. The authors showed the inhibitory effect of *E. plana*'s fresh biomass on *P. notatum* germination (Ferreira et al. 2008). Furthermore, most of the studies related to *E. plana* and *C. dactylon* phytotoxicity have alternatively used cultivated species as recipients (e.g., *Lactuca sativa, Trifolium repens,*

Lycopersicon esculentum). These species have been used for being sensitive to allelopathic compounds, present rapid germination and uniform initial growth (Reigosa et al. 2013). However, according to the well-known novel weapon hypothesis, invader's allelochemicals have been considered a new mechanism of interaction in the introduced range, as neighbors may not have co-evolved to resist inhibition, which may enhance invasion success (Callaway and Aschehoug 2000; Ridenour and Callaway 2001; Callaway and Ridenour 2004; Inderjit et al. 2011). Thus, for testing allelopathy in laboratory conditions, the inclusion of a recipient species, that may be naturally associated with the invader in the introduced range, could help to generate more applicable data to field conditions to understand the underlying mechanisms of invasion.

We would have expected that both donor invasive species cause greater inhibitory effects than the co-occurring native, assuming the invasive species may have phytochemicals with harmful effects to enhance invasiveness (Callaway and Aschehoug 2000; Ridenour and Callaway 2001; Callaway and Ridenour 2004; Inderjit et al. 2011). However, our data did not support this statement. The invasive *C. dactylon* showed the strongest inhibitory effect on *E. bahiensis* germination but, interestingly, the native *C. selloana* had greater negative effect than the well-studied invasive grass *E. plana*. Regarding the early growth of seedlings, *C. dactylon* and *C. selloana* had both similar negative effects on the emergence of leaves, and this effect was significatively stronger than *E. plana´s*. Thus, *E. plana*, which many studies have suggested that presents high allelopathic potential (Coelho 1986; Ferreira et al. 2008; Favaretto 2011, 2015, 2019; Cecchin et al. 2017; Bittencourt et al. 2018a,b; Scheffer-Basso 2019), was the donor species with the minor inhibitory effect. Including a donor native species as a species origin control was crucial for disentangle the relative effect of both invasive species. This consideration has been already suggested in other experimental designs for testing and relativizing the invasive species impact (Vilà and Weiner 2004; Del Fabbro et al. 2013; Dresseno et al. 2018; Guido et al. 2019), but it is still often ignored. In our experiment, the donor native species provided a comparison about how novel was the effect of the invasive species (i.e., phytotoxicity) in the recipient community (i.e., introduced range). None

of the previous phytotoxicity studies about *C. dactylon* and *E. plana* invasion have used a donor native species. Systematic reviews have shown that most allelopathy studies with extracts demonstrated negative effects of one species to another; neutral effects have been rarely reported (Reigosa et al. 2013; Silva et al. 2017). Thus, we consider it is important to relativize the effect of the invader in the recipient community by considering a species origin control. Complementary, studies that identify donor species compounds related to phytotoxicity are also needed for testing chemical novelty of invasive species in the introduced range. Favaretto et al. (2018, 2019) described *E. plana* compounds but there is not a phytochemical analysis carried out for *C. dactylon* in our study region, and none information is available for *C. selloana*. We encourage researchers to better understand plant-plant interactions in the recipient community for improving the understanding about the novel effects of *C. dactylon* and *E. plana* in Río de la Plata grasslands.

In conclusion, we highlight the importance of using an adequate post-harvesting method, a native recipient species in the introduced range and suitable experimental controls. This approach may help understanding the role of allelopathy in the complex invasion process of natural ecosystems. Our results indicated that leachates of the standing fresh leaves of *C. dactylon* and *E. plana* may not inhibit neighboring species in Río de la Plata grasslands. However, it would be important to validate these results at field conditions, as caution should be taken when extrapolating laboratory results to explain processes in the field (e.g., Del Fabbro et al. 2013). Moreover, all extracts from dried leaves were phytotoxic, but this post-harvesting method could be far from representing a real field condition (i.e., compounds concentration), although it was the most used method in the literature for suggesting the allelopathic potential of *E. plana*. We are aware our experiment had some limitations that should be improved in further studies, such as the short-term observation period to assess the effects on plant growth, the low number of donor species (only one native and two invasive plants) and only one recipient species. However, we do consider our results are appropriate to raise questions about the role of phytotoxicity of *C. dactylon* and *E. plana* invasion. Our considerations could be useful for further studies, by showing the importance of using native species as donors

for relativizing the invasive species effect and by using native species as recipients for testing novelty in the introduced range.

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