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Phenolic exudates from *Ludwigia peploides* and *Azolla* sp. enhance germination of *Polygonum ferrugineum* seeds

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ABSTRACT

The release of allelochemicals by aquatic macrophytes can inhibit or benefit other plants. Here we studied allelopathic interactions between syntopic species native to the Neotropical region. First, we quantified the phenolic exudation in monospecies cultures of *Ludwigia peploides* (L) and *Azolla* sp. (A) and in co-cultures of both species growing together (A + L) compared to control water. Then, we studied the germination of *Polygonum ferrugineum* seeds exposed to the exudates and control water. Incubation water from L had a significant lower pH than the other treatments, and a lower conductivity than the control. L alone or in co-culture with A reduced the dissolved oxygen content of the incubation water. Phenolic compounds were undetectable in control water (C) and the A treatment. In water of L and A + L treatments, phenolic concentration increased during the 5-day bioassay, with significant differences from day 3 on compared to treatments C and A. On day 5, more phenolic exudates were found in the A + L than in the L treatment. The germination rate but not germination speed was significantly higher in *P. ferrugineum* seeds treated with A + L exudates, and correlated positively with the phenolic concentration. Our results suggest that the allelopathic potential of aquatic macrophytes such as *L. peploides* can be higher in multispecies patches than in monospecies ones. Furthermore, phenolic exudates of macrophytes might stimulate the germination of plants native to the same region, which may have implications in the formation of vegetation patterns in aquatic ecosystems.

1. Introduction

Aquatic macrophytes are key components of freshwater ecosystems (Neiff, 1986; Wetzel, 2001). They influence nutrient cycling (Esteves, 1998) and sediment dynamics (Madsen et al., 2001), increase environmental heterogeneity (Taniguchi et al., 2003; Thomaz et al., 2008) and provide habitat and food for many organisms such as periphyton, invertebrates and fishes (Agostinho et al., 2007; Thomaz et al., 2008; Pettit et al., 2016). Through the release of allelochemical substances into the surrounding water, macrophytes can inhibit or stimulate autotrophs (Gross, 2003; Hilt and Gross, 2008).

The allelopathic phenomenon in aquatic plants has received increasing attention in the last decades (Gross, 2003; Dandelot et al., 2008; Xu et al., 2018) partly due to the possible application of allelopathic plants or their allelochemicals in the management of aquatic systems (Hilt and Gross, 2008), such as the control of undesired aquatic weeds (Willis, 2008) or algal blooms (Gross and Sütfeld, 1994; Gross et al., 1996; Pakdel et al., 2013), and the regulation of vegetation patterns (Rice, 1979; Erhard, 2006). However, most studies have typically centered on a few agricultural target plants, which do not coexist with the donor plants under natural conditions (Gawronska and Golisz, 2006; Reigosa et al., 2013). From an ecological point of view, the latter is questionable because aquatic plants do not compete with plants such as lettuce or cress in their natural habitats (Erhard, 2006).

Several emergent and free-floating plants native to Neotropical regions are considered to have a high allelochemical potential, even within their native distribution ranges (Gutierrez and Mayora, 2015; Grutters et al., 2017), and the release of allelopathic compounds could affect other co-occurring native macrophytes. Particularly, the genus *Ludwigia* has been widely studied because of its high phenolic content and exudation in comparison with other macrophytes (Gutierrez and Mayora, 2015; Grutters et al., 2017), which might partly explain its high invasive potential (Dandelot et al., 2008). However, it is also judicious to investigate the allelopathic potential of species of this genus against syntopic plants native to the same region (Erhard, 2006), but this has received little attention so far.

Many biotic and abiotic factors may influence the production and release of allelopathic compounds; for example, the presence of neighbor species, resource availability and integrity of macrophyte tissues (Gopal and Goel, 1993; Gawronska and Golisz, 2006; Gross

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et al., 2012; Gutierrez and Mayora, 2015). Gopal and Goel (1993) have claimed that the allelopathic potential of a species can be enhanced by the presence of a competing species. Furthermore, it has been suggested that the production of allelopathic compounds is inducible and that an individual plant will produce more allelochemicals when environmental conditions, such as limited space, light or nutrient, trigger their synthesis (Reigosa et al., 1999a; Gawronska and Golisz, 2006). In this sense, macrophytes might increase phenolic exudation when occurring in multispecies patches because of increasing competition by resources.

Detrimental effects of allelopathic substances such as delaying germination, affecting seedling development and inhibiting the growth of plants, are more common than beneficial ones (Erhard, 2006; Dandelot et al., 2008). Allelopathic effects on germination are highly variable because they are species-specific and depend on the nature and concentration of the allelopathic substances involved, as well as on other environmental variables (Reigosa et al., 1999a).

Phenolic compounds are a major class of secondary metabolites involved in plant allelopathy (Appel, 1993; Erhard, 2006; Reigosa et al., 2013). In South America, the emergent *Ludwigia peploides* (Kunth) P.H. Raven and the free-floating *Azolla filiculoides* Lam. and *A. cristata* Kaulf. are native plants widespread in wetlands such as the Paraná River floodplain (Schneider et al., 2015) and are both involved in allelopathic interactions (Gutierrez and Mayora, 2015). Thus, *L. peploides* and *Azolla* sp. may affect other native macrophytes. However, according to our knowledge, the allelopathic potential of these species has not been studied within their natural distribution.

We thus aimed 1) to evaluate the phenolic exudation in monospecies cultures of *L. peploides* and *Azolla* sp., and in a culture with both species growing together, and 2) to evaluate the relation between the exudation of phenolic compounds and germination patterns of seeds of the native emergent *Polygonum ferrugineum* Wedd. We chose *P. ferrugineum* because this macrophyte usually co-occurs with *L. peploides* and *Azolla* sp. and produces numerous seeds with high germination potential (Montenegro et al., 2006). We hypothesized that 1) the coexistence of *L. peploides* and *Azolla* sp. favors their phenolic exudation; and 2) the phenolic exudates negatively affect seed germination of *P. ferrugineum*.

2. Materials and methods

2.1. Plant materials

The exudates were obtained from mature and healthy plants of *Ludwigia peploides* (Onagraceae) and *Azolla* sp. (Azollaceae) manually collected in summer (December 2014). Emergent individuals of *L. peploides* with approximately 30 cm in height, were randomly collected from aquatic macrophyte patches of a wild population in a shallow lake (31°37′S, 60°41′W, Argentina), and *Azolla* sp. plants were randomly collected from an outdoor tank located in the riparian zone of the lake containing rain water and a culture generated by the Instituto Nacional de Limnología. Plants were transported to the laboratory in plastic containers where they were carefully washed with tap water and, finally, with distilled water to remove attached sediment and invertebrates.

Although the dispersal unit of *Polygonum* is a seed covered by adhering fruit structures (achene), we use the term *seed* to represent this unit. Mature seeds of *P. ferrugineum* were manually collected during summer (November 2014) directly from plants from a natural population of an anabranch of the Middle Paraná River (31°49′ S, 60°36′ W) before natural dispersion occurred.

2.2. Experimental design

We performed two bioassays (Fig. S1). Bioassay 1 was performed to evaluate the phenolic exudation in monospecies and multispecies cultures of *L. peploides* and *Azolla* sp. We used either *A. filiculoides* or *A. cristata,* but we were not able to identify the used species. Bioassay 2 was conducted to evaluate the relation between the phenolic compounds exudated by these cultures (obtained in bioasssay 1) and the germination rate and speed of *P. ferrugineum* seeds exposed to the exudates.

Bioassay 1 was carried out in summer (January 2015) in plastic cubic containers with 10 cm in height and a surface of 324 cm^2. Each container was filled with 1 L of dechlorinated tap water, and daily enriched with KH_2PO_4 (final concentration: 5 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (36 mg L^{-1}), $Ca(NO_3)_2{\cdot}4H_2O$ (36 mg L^{-1}), KCl (5 mg L^{-1}), and NH_4Cl (0.8 mg L^{-1}). The experimental design included a control (C: container with water enriched with nutrients but without plant material) and three treatments (A: a monoculture of *Azolla* sp., L: a monoculture of *L. peploides*, and A + L: a culture of both species growing together). Each treatment and the control were replicated three times (three independent plastic containers), resulting in 12 independent replicates (four containers repeated three times each) (Fig. S1). To minimize variances due to the effects of differences in wet plant biomass, plant material was weighed and 60 g of *L. peploides* (three to four individuals; treatments L and A + L) and 40 g of *Azolla* sp. (~180 individuals; treatments A and A + L) were placed in each container. The percentage cover of *Azolla* sp. and *L. peploides* plants was ~ 100% and ~ 25%, respectively. Each replicate was maintained indoor for 5 days under natural photoperiod conditions (allowed by the presence of transparent surfaces connected with the external environment) and at constant environmental temperature of ~25 °C.

In each replicate, temperature, pH, conductivity, and dissolved oxygen were daily measured with Hanna portable probes. In addition, one sample of water per treatment replicate was daily collected using glass bottles. Samples were filtered through membrane filters (0.45 μm pore size) to determine phenolic compound concentration using the Folin–Ciocalteu reagent (Box, 1983), which gives a rough approximation of the total phenolic content of a sample (Everette et al., 2010). Sample absorbance was measured at 765 nm and expressed as gallic acid equivalents based on a calibration curve with this compound. In addition, phenolic content was normalized by total wet weight of plants in each treatment. Once the assay was complete, the culture media and the control water of each replicate were separately collected and kept for one week refrigerated at 4 °C in dark glass bottles in order to use them in the germination bioassay (bioassay 2).

In bioassay 2, the culture media of the plants and control water obtained in the previous study were used to test the allelopathic potential of the exudates on the germination of *P. ferrugineum* seeds. Prior to the bioassay, the persistent perianths were removed and uniform seeds were selected (approximately 3 mm × 3 mm) and stratified in dechlorinated tap water at 10 °C for 1 week until use. We used this stratification method because it was effective for *P. ferrugineum* in subtropical ecosystems (Benech Arnold, pers. comm.) as well as for several species of the genus *Polygonum* in other latitudes (Hock et al., 2006; Khan and Ungar, 1998; Timson, 1965). Empty seeds that remained afloat were discarded, and only those which sank (and were potentially viable) were used in the assay (Varela and Arana, 2011). Seeds were randomly assigned to 60 groups of 20 seeds each (total quantity of seeds = 1200). Each seed group was arranged on two qualitative filter papers No. 102 (Producer: New Star, Origin: China) placed in the base of a sterile Petri dish (9 cm diameter), ensuring that all seeds had the same degree of contact with the substrate. Filter papers were previously treated with ethanol to prevent fungal and bacterial growth. Ethanol was allowed to evaporate before the beginning of the assay. Petri dishes were allotted in completely randomized design and assigned to 12 groups of five (one group for each replicate of bioassay 1), resulting in 60 independent replicates (Fig. S1). Petri dishes of each group received 8 mL of culture medium or control water corresponding to 1 replicate of the previous experiment. The Petri dishes were closed with dish covers to reduce water loss by evaporation and were stored in an incubator at 10 °C with a photoperiod of 12:12 h L:D and 135 μmol photons PAR m^{-2} s^{-1}. We used this temperature

because it showed the highest germination rates in previous studies with *P. ferrugineum* (Benech Arnold, pers. comm.). During the incubation, Petri dishes were daily opened to allow air exchange and to count germinated seeds (visible emergence of the radicle from the seed). When necessary, 2–4 mL of culture media or control water were added to keep the filters wet. Culture media and control water were maintained refrigerated throughout the germination bioassay, which lasted for 37-days and ended when no additional seeds germinated for seven consecutive days.

For the evaluation of the relation between the variable phenolic concentration and seed germination, the accumulated germination rate (% G) (1) and the germination speed (GS) (2) were calculated as:

$$\%G = G(100)/S_T \quad (1)$$

where G is the accumulated number of germinated seeds at the end of the bioassay and S_T is the total number of seeds in each replicate,

$$GS = \sum_{i=1}^{n} N_i G_i \Big/ \sum_{i=1}^{n} G_i \quad (2)$$

where *Ni* is the number of days since the beginning of the bioassay and *Gi* is the number of germinated seeds at day *i* (Nakagawa, 1999).

2.3. Statistical analysis

Statistical analyses were performed using PAST 3.18 software (Hammer et al., 2001). A repeated measure one-way ANOVA was used to evaluate temporal changes in the measured variables in culture water (A, L and A + L) and control water (C). A one-way ANOVA was performed to test for significant differences between culture water (A, L and A + L) and control water (C) on each experimental day (1, 2, 3, 4 and 5). In addition, this analysis was used to test for significant differences in germination speed and final rate of *P. ferrugineum* between treatments and control. The pairwise differences were analyzed using Tukey post hoc test. Data were tested for homogeneity of variances using Levene's test based on medians. Spearman correlations were performed to evaluate the relations between the concentration of phenolic compounds in each replicate of treatments and control (first bioassay) and the germination speed and accumulated germination rate (at the end of the experiment) of *P. ferrugineum* seeds (average values of the five replicates of the second bioassay).

3. Results

Plants maintained their healthy state throughout the bioassay 1 (phenolic exudation). At the beginning of the bioassay, treatments and control showed similar values of temperature, conductivity, pH, and dissolved oxygen, whereas the concentration of phenolic compounds was undetectable in all replicates (one-way ANOVA: df = 3, $p > 0.05$). Temperature remained constant throughout the bioassay (22.0 ± 0.3 °C, repeated measures one-way ANOVA: df = 4, $p > 0.05$).

Conductivity remained constant in control water and treatment A + L (mean ± SD: 1208 ± 89 μS cm^{-1}, 1060 ± 40 μS cm^{-1}, respectively, repeated measures one-way ANOVA: df = 4, $p > 0.05$). In contrast, conductivity decreased throughout the bioassay in A and L treatments (repeated measures one-way ANOVA: df = 4, F = 19.55, F = 54.52 respectively, $p < 0.05$). At the end of the experiment (day 5), values were significantly higher in control water than in A, L and A + L treatments (Table 1, Fig. 1A). The pH increased in all treatments with time. Control water differed significantly from L and A + L starting from day 2 (Table 1, Fig. 1B). Dissolved oxygen decreased in all treatments with time. Significant differences existed between treatments with and without *L. peploides* starting with day 2 (Table 1, Fig. 1C).

Phenolic compounds were undetectable along the experiment in control water and treatment A. In treatments L and A + L, the concentration of phenolic compounds increased with time (Fig. 2A), showing significant differences compared to control water and treatment A starting with day 3 (Table 1). The increase in concentration of phenolic compounds was more marked on day 5 in treatment A + L than in treatment L (Table 1). When comparing the quantity of phenolic compounds exudated per plant biomass, the A + L treatment also showed the highest values, with significant differences to the A and L treatments at day 5 (Table 1, Fig. 2B).

At the end of the bioassay 2 (seed germination), germination rates were ≥ 75% in all treatments, and ≥ 65% in the controls. Germination started on the eighth day in all treatments and control without significant differences in speed (one-way ANOVA: df = 3, F = 1.14, $p > 0.05$). However, the germination rate showed significant differences among treatments (one-way ANOVA: df = 3, F = 3.69, $p < 0.05$), with the highest values in seeds treated with A + L exudates (Fig. 3). In addition, the germination rate was positively correlated with the concentration of phenolic compounds (Spearman correlation, rho = 0.61, $p < 0.05$, Fig. 4). In contrast, the germination speed was not associated with this variable (Spearman correlation, rho = 0.00, $p > 0.05$).

4. Discussion

As predicted, total exudation of phenolic compounds was higher in the multispecies culture of *L. peploides* and *Azolla* sp. than in the monospecies cultures of both species; thus, our first hypothesis was validated. However, contrary to expectations, the phenolic exudates positively affected seed germination of *P. ferrugineum*; thus, we could not accept our second hypothesis.

4.1. Exudation of phenolic compounds by Azolla sp. and L. peploides

The high phenolic exudation of *L. peploides* is in accordance with previous bioassays (Gutierrez and Mayora, 2015) and with measurements made in macrophytes collected in the Middle Paraná system (Mayora, pers. obs.). According to these studies, *L. peploides* showed the highest phenolic exudation and content (mean value: 150 mg g^{-1} DW) when compared to other abundant species. In contrast, the undetectable concentration of phenolic compounds in exudates of *Azolla* sp. is in contrast to the mentioned studies, according to which this fern has a considerable phenolic exudation and content (mean value: 29 mg g^{-1} DW). However, due to the important variability in the phenolic content of *Azolla* sp. (2–68 mg g^{-1} DW) (Mayora, pers. obs.), it can be expected that its exudation also vary widely.

Competition for resources and stress conditions may influence the production and exudation of phenolic compounds by aquatic macrophytes (Cronin and Lodge, 2003; Reigosa et al., 1999a). We supplied nutrients in excess but light and space may have been limiting resources, mainly in the multispecies culture of *L. peploides* and *Azolla* sp. because plant cover was higher than 100%. A higher level of exudation of phenolic compounds in the multispecies culture than in the monospecies ones could be a response to a greater competition for space since these metabolites may be detrimental to potentially competing macrophytes (Ervin and Wetzel, 2003; Gopal and Goel, 1993). Although it is impossible to know whether *Azolla* sp., *L. peploides* or both increased the exudation of phenolic compounds in the multispecies culture in comparison with the monospecies ones, the results show the importance of ecological factors, particularly that of species coexistence, for phenolic exudation (Ervin and Wetzel, 2003). Despite some limitations in the extrapolation of our experimental results to natural conditions, our work highlights the importance of using multispecies cultures to evaluate phenolic exudation since vegetated patches in field are rarely monospecies.

Table 1
Results of one-way analysis of variance (ANOVA, on the left of the table) and Tukey's pairwise comparisons (on the right of the table) performed to assess significant differences among control water and water from the different mono- and multispecies cultures with time (bioassay days). C: control water; culture water of A: *Azolla sp.*, L: *L. peploides*, A + L: both species growing together. *Normalized by plant biomass.

Variable		One-way ANOVA			p value – Tukey's comparisons		
	Day	df	F	p		L	A + L
Phenolic compound	3	3	19.7	< 0.001	C	< 0.05	< 0.01
					A	< 0.05	< 0.01
	4	3	7.1	< 0.05	C		< 0.05
					A		< 0.05
	5	3	64.2	< 0.001	C	< 0.05	< 0.01
					A	< 0.05	< 0.01
					L		< 0.01
Phenolic compound exudation*	3	2	7.5	< 0.05	A	< 0.05	< 0.05
	5	2	20.2	< 0.01	A		< 0.01
					L		< 0.05
Conductivity	2	3	5	< 0.05	C		< 0.05
	4	3	10.6	< 0.01	C		< 0.01
					L		< 0.05
	5	3	5.5	< 0.05	C	< 0.05	
pH	2	3	6.2	< 0.05	C	< 0.05	< 0.05
	3	3	14.9	< 0.001	C	< 0.01	
					A	< 0.001	< 0.05
					L		< 0.05
	4	3	6.3	< 0.05	C		< 0.05
					L		< 0.05
	5	3	14.1	< 0.001	C	< 0.01	
					A	< 0.01	
					L		< 0.05
Dissolved oxygen	2	3	11.3	< 0.01	C	< 0.05	
					A	< 0.01	< 0.05
	3	3	51.6	< 0.0001	C	< 0.001	< 0.001
					A	< 0.001	< 0.001
	4	3	22.8	< 0.001	C	< 0.05	< 0.001
					A		< 0.01
					L		< 0.05
	5	3	104.6	< 0.0001	C	< 0.001	< 0.001
					A	< 0.001	< 0.001
					L		< 0.01

4.2. Effect of phenolic exudates on seed germination in P. ferrugineum

Phenolics are an extremely diverse group of compounds with different modes of action, and different ecological activities (Appel, 1993; Reigosa et al., 1999b). Macrophytes are known to release phenolic compounds that have the ability to affect their competitors, in many cases, through the inhibition of seed germination (Dandelot et al., 2008; Reigosa et al., 1999b). However, contrary to what was expected, the germination rate increased with the phenolic concentration whereas the germination speed was not affected by exudates. Positive allelopathic interactions have been scarcely observed (Gross, 2003) and thus our study highlights the need to investigate more deeply this kind of interaction.

There are at least three possible explanations for the positive association between the phenolic concentration and the germination rate. Firstly, it has been suggested that phenolic compounds have low toxicity and typically generate positive responses in physiological processes at low concentrations, such as frequently found under natural conditions (Reigosa et al., 1999a). Given that in our experiment exudates were obtained directly from recipients with *L. peploides* and *Azolla* sp. in a density similar to that found in field, we expected a phenolic concentration similar to that naturally occurring in aquatic habitats, and this could have been low enough to generate a beneficial effect on germination. A different result could occur at a phenolic concentration exceeding the level of tolerance for seed germination (Reigosa et al., 1999a). This is more likely to occur in experiments using phenolic extracts, which is the most common experimental approach for allelopathic interferences (Hilt and Gross, 2008).

Secondly, because of their reactivity and acidic properties (Vermerris and Nicholson, 2009), phenolic compounds could reduce the thickness and augment the porosity of seed teguments, favoring water imbibition and, therefore, germination (Tobe et al., 2001). Values of pH at the end of the exudation bioassay were slightly lower in treatments with higher concentration of phenolic compounds. However, given that the pH scale is logarithmic, low differences in pH represent higher differences in acidity. For example, the difference in acidity due to hydrogen ions was 62% between the L treatment (final pH = 6.49 ± 0.1) and control (final pH = 6.70 ± 0.1).

And thirdly, the degradation of phenolic compounds by microorganisms decreases the concentration of dissolved oxygen (Mendonça et al., 2004). This is in accordance with the lower concentration of dissolved oxygen in culture water with higher phenolic concentration. Seeds of some aquatic species reach high germination rates only under the low oxygen levels associated with flooding (Baskin and Baskin, 2014). Thus, the low levels of dissolved oxygen might have promoted seed germination in L and A + L treatments. Indeed, it has been shown that lack of oxygen often breaks dormancy in seeds requiring cold stratification (Come et al., 1991). However, the conclusion of this author was based on seeds of terrestrial species and whether the same occurs in aquatic plants should be tested.

A suitable temporal distribution of seed germination is critical for the survival and development of seed plants. If speed increases and seeds germinate altogether, seedlings could be affected by competition; while if speed is delayed, germination of most seeds could occur under unfavorable conditions (Neé et al., 2017). In our study, germination speed was not affected by the phenolic exudates. It has been suggested that negative allelopathy occurs mainly between plants native to different regions, and that the influence of allelopathic interactions may

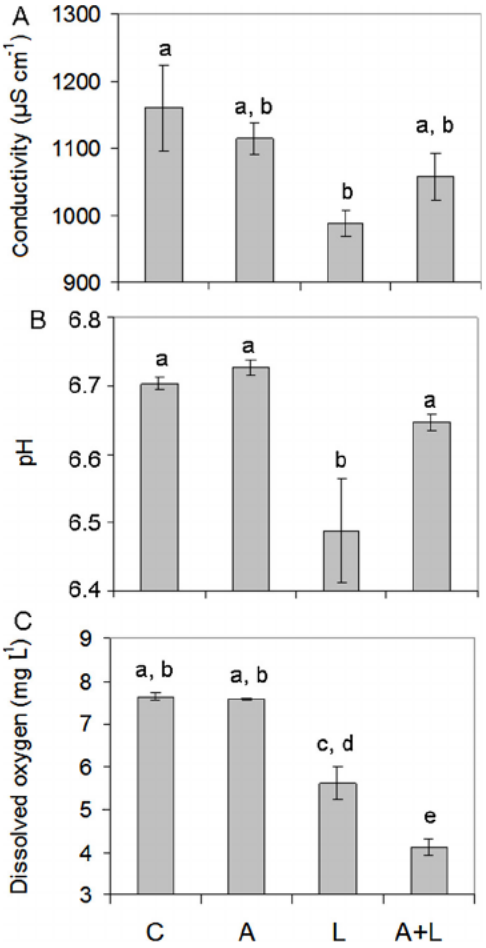

Fig. 1. Mean values of conductivity (A), pH (B) and dissolved oxygen (C) in the control water and the culture water of plants at the end of bioassay 1 (day 5). Error bars are mean absolute deviations, n = 3. Different letters indicate significant differences according to one-way ANOVA and Tukey's pairwise comparisons ($p < 0.05$). C: control water; culture water of A: *Azolla sp.*, L: *L. peploides*, A + L: both species growing together.

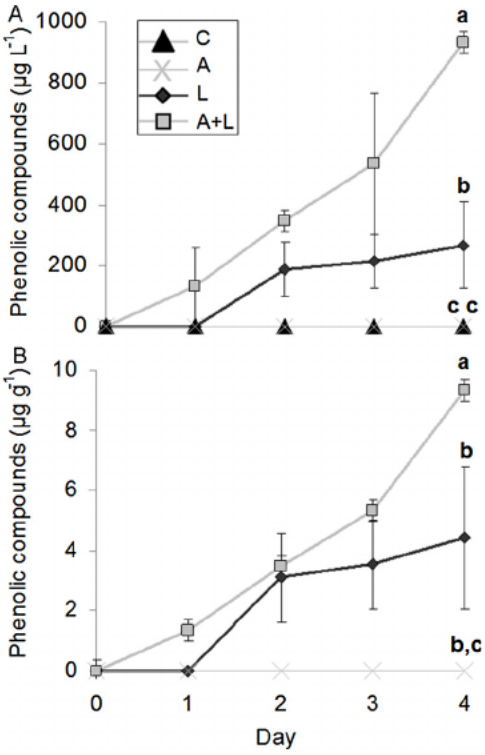

Fig. 2. Variation in concentration of phenolic compounds in the control water and the culture water that contained the studied macrophytes during 5 days (A), and quantity of exudated phenolic compounds normalized by initial total wet weight of plants (B). Error bars are mean absolute deviations, n = 3. C: control water; culture water of A: *Azolla sp.*, L: *L. peploides*, A + L: both species growing together.

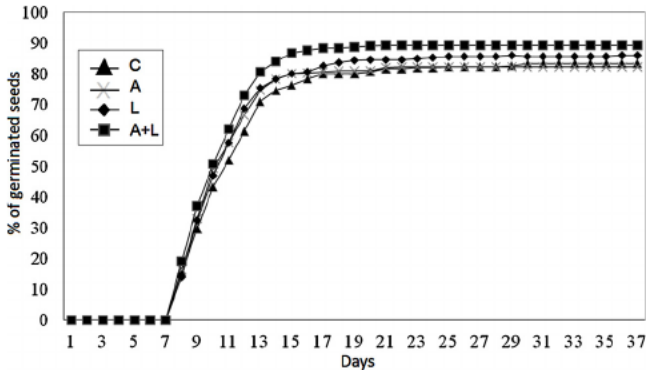

Fig. 3. Accumulated germination rate of exudate-treated seeds and control during the 37-day experiment (bioassay 2). C: control water; culture water of A: *Azolla sp.*, L: *L. peploides*, A + L: both species growing together.

be more pronounced in special situations, as when an alien plant invades an established plant community (Reigosa et al., 1999a; Erhard, 2006). For instance, in other latitudes, alien *L. peploides* exudates were shown to negatively affect the germination of native plants with which it did not co-evolve (Dandelot et al., 2008). In contrast, co-occurring species were suggested to be less susceptible to allelopathy than species from foreign habitats (Reigosa et al., 1999a; Gross et al., 2012). *Polygonum ferrugineum*, which usually coexists with *L. peploides* and *Azolla* sp., might have developed resistance or tolerance in response to the phenolics it is exposed to, even gaining benefit from them through the previously mentioned mechanisms and/or others, whose elucidation requires more research.

5. Conclusions

We obtained exudates from species that coexist within their natural habitats and we used high plant densities similar to those observed in natural ecosystems associated to the Paraná River to reflect the allelopathic activity of phenolic exudates occurring in nature. However, in a natural system, macrophyte patches are often composed by more than two species, and it is probable that a different situation from the one discussed in this study may arise. Nevertheless, from this experimental research, we can state that phenolic exudation of native macrophytes can increase due to their coexistence in a same habitat and that these exudates can favor the germination of other native aquatic species. This suggests that phenolic compounds exudated by native plants can be important in the formation of vegetation patterns in aquatic ecosystems.

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Declaration of interest

The authors confirm that this article content has no conflict of interest.

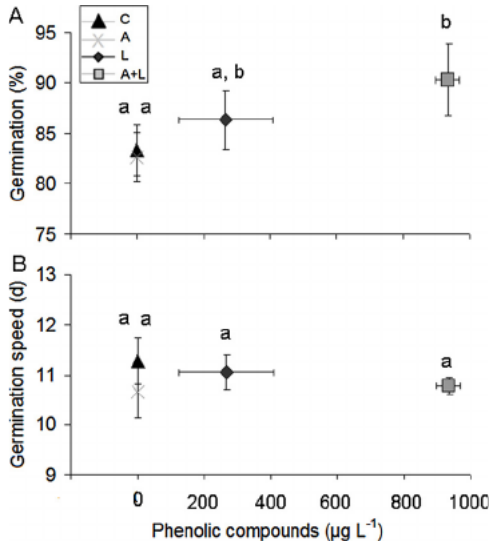

Fig. 4. Relation of the concentration of phenolic compounds to the germination rate (A) and germination speed (B) of seeds treated with control water and plant exudates. Horizontal and vertical error bars mean absolute deviations; n = 3 and 15, respectively. Different letters indicate significant differences in germination according to one-way ANOVA and Tukey's pairwise comparisons ($p < 0.05$). C: control water; culture water of A: *Azolla sp.*, L: *L. peploides*, A + L: both species growing together.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aquabot.2018.08.005.

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