Allelopathy of aqueous *Pachyrhizus erosus* L. extracts on *Euphorbia heterophylla* and *Bidens pilosa*[1]

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ABSTRACT

Allelopathy contributes to the sustainable management of weeds in growing areas, due to its ability to inhibit the development of weed species. This study aimed to evaluate the allelopathic effect of *Pachyrhizus erosus* extract on *Euphorbia heterophylla* and *Bidens pilosa* germination. The experiment was carried out in a completely randomized design, in a 2 x 2 x 4 factorial scheme, with four replications. The first factor corresponded to the plant portion (shoot and tuberous root), the second to the extract preparation method (water at room temperature and boiling) and the third to the extract concentrations (0 %, 25 %, 50 % and 100 %). Phytochemical analyses were performed to identify the presence of secondary metabolites. The variables germination percentage, germination rate index and dry biomass decreased with increasing aqueous extract concentrations for both *E. heterophylla* and *B. pilosa.* When applied a *P. erosus* crude shoot extract (boiling), decreases of 18 % and 92 % were observed in the *E. heterophylla* and *B. pilosa* germination, respectively, with a decrease of 91 % in the dry biomass of *E. heterophylla* seedlings. The tuberous root extract (boiling), on the other hand, reduced the germination percentages for *E. heterophylla* and *B. pilosa* in 18 % and 80 %, respectively. The phytochemical evaluation indicated the presence of flavonoids, coumarins and saponins in the shoot and tuberous root extracts, while tannins were only observed in the shoot extract. Thus, it can be concluded that *P. erosus* extracts display an allelopathic effect on the weed species evaluated.

KEYWORDS: Yam bean; weed; wild poinsettia; beggartick.

RESUMO

Alelopatia do extrato aquoso de *Pachyrhizus erosus* L. sobre *Euphorbia heterophylla* e *Bidens pilosa*

A alelopatia contribui com o manejo sustentável de plantas daninhas em áreas de cultivo, devido à capacidade de inibir o desenvolvimento de espécies infestantes. Objetivou-se avaliar o efeito alelopático do extrato de *Pachyrhizus erosus* na germinação de *Euphorbia heterophylla* e *Bidens pilosa*. Utilizou-se delineamento inteiramente casualizado, em fatorial 2 x 2 x 4, com quatro repetições. O primeiro fator correspondeu à parte da planta (parte aérea e raiz tuberosa), o segundo ao método de preparo (água à temperatura ambiente e em ebulição) e o terceiro à concentração dos extratos (0 %, 25 %, 50 % e 100 %). Análises fitoquímicas foram realizadas para identificar a presença de metabólitos secundários. As variáveis porcentagem de germinação, índice de velocidade de germinação e biomassa seca reduziram-se conforme o aumento da concentração dos extratos aquosos, tanto para *E. heterophylla* quanto para *B. pilosa*. Houve 18 % e 92 % de redução na germinação de *E. heterophylla* e *B. pilosa*, respectivamente, quando utilizado o extrato bruto da parte aérea (ebulição) de *P. erosus*, com redução, ainda, de 91 % da biomassa seca das plântulas de *E. heterophylla*. O extrato da raiz tuberosa (ebulição) reduziu em 18 % e 80 % a porcentagem de germinação de *E. heterophylla* e *B. pilosa*, respectivamente. A avaliação fitoquímica indicou a presença de flavonoides, cumarinas e saponinas para o extrato da parte aérea e da raiz tuberosa, enquanto taninos foram observados apenas no extrato da parte aérea. Conclui-se que os extratos preparados com *P. erosus* possuem efeito alelopático nas espécies avaliadas.

PALAVRAS-CHAVE: Jacatupé; planta daninha; leiteiro; picão-preto.

INTRODUCTION

Plant species may stimulate or inhibit the development of other species by the action of secondary metabolism products, which, when released into the environment, cause morphological and metabolic changes in neighboring species (Fiorenza et al. 2016). The allelopathic action of substances produced by plants has aroused interest, regarding the product development of compounds that can be applied as natural herbicides and reduce economic damages caused by weeds in the agricultural environment (Maciel et al. 2017).

Substances derived from the secondary metabolism of plants are present in different amounts, depending on the plant organ, and may be toxic to

1. Manuscript received in Jan./2018 and accepted for publication in Mar./2018 (http://dx.doi.org/10.1590/1983-40632018v4851117).
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other species during different developmental periods (Khaliq et al. 2012). For example, the germination percentage of *Lycopersicum esculentum* was shown to decrease over 80 %, when applying a dry *Duranta repens* extract, while the extract prepared with the fruits of this species reduced the same variable by only 20 % (Tur et al. 2010). In another study, a *Pouteria ramiflora* bark extract did not influence lettuce seed germination, although primary root length was reduced by up to 81 % (Oliveira et al. 2014).

Substances present in plant material can be extracted by different methods, leading to differences in yield. For example, a crude *Mimosa tenuiflora* (jurema-preta) extract prepared with water at 100 ºC reduced *L. sativa* germination by 7.5 %, but the same extract prepared at 25 ºC only reduced germination by 2.5 % (Silveira et al. 2012). In another study, Lessa et al. (2017) also observed that *Plectranthus barbatus* extracts led to better results in inhibiting *Amaranthus deflexus* germination, when prepared by infusion (water at 100 ºC), leading to a germination decrease of 86 %, if compared to the controls.

The *Pachyrhizus* spp. genus comprises three neotropical cultivated species (*P. erosus*, *P. ahipa* and *P. tuberosus*) belonging to the Fabaceae family. It is one among many plants that contain toxic compounds, such as astoxic polyphenols (rotenone and pachirzine), as reported by Balbin et al. (2007) and Lautié et al. (2013). However, little is known about the allelopathic effects of this plant species on weeds.

Allelopathic substances present in the secondary metabolism of plant species may alter important metabolic pathways active in the germination process and early seedling development. Therefore, the hypothesis of this study is based on the fact that *P. erosus* aqueous extracts obtained from the shoots and tuberous roots of this plant, prepared in different manners, will exhibit negative allelopathic effects on *Euphorbia heterophylla* and *Bidens pilosa* germination.

Therefore, this study aimed to evaluate the allelopathic effects of *P. erosus* aqueous extracts obtained from the shoots and tuberous roots of this plant, in different concentrations, on *E. heterophylla* and *B. pilosa* germination.

MATERIAL AND METHODS

The experiments were carried out under laboratory conditions at the Universidade Estadual do Oeste Paranaense, in Marechal Cândido Rondon, Paraná state, Brazil. *P. erosus* shoots, comprising leaves and stems, and tuberous roots were obtained from plants in the pre-flowering period, from an experimental area (24º42'30"S, 54º21'10"W), in May 2017. The tuberous roots were sectioned and the plant material maintained in a forced ventilation oven (65 ºC) until constant mass. The dry material was stored away from light and moisture.

The dry plant material was then diluted in both distilled water at room temperature (25 ºC ± 3) and in boiling water (100 ºC) at 8 % (w/v) concentration, and homogenized with a glass rod. The extracts were left resting for 24 h in the absence of light and in cool conditions (± 10 ºC). Subsequently, a vacuum filtration was carried out, resulting in the crude extracts of each plant portion (100 %), which were applied soon after preparation.

Qualitative phytochemical analyses of the crude extracts were performed to identify secondary metabolites in each plant portion. Compound detection was carried out by specific reactions (presence or absence), according to a methodology adapted from Simões (2010). Four groups of secondary metabolites (flavonoids, coumarins, saponins and tannins) were analyzed.

Regarding the germination test, the design was completely randomized, in a 2 x 2 x 4 factorial scheme, with four replications. The first factor corresponded to the plant portion (shoot and tuberous root), the second to the way the extracts were prepared (water at room temperature and boiling water) and the third to the aqueous extract concentrations (0 %, 25 %, 50 % and 100 %). Each replicate comprised 25 commercially obtained *E. heterophylla* seeds and 25 *B. pilosa* seeds collected from a rural area in the west Paraná region.

The seeds were packed in Gerbox-type plastic boxes, with two germitest paper sheets previously sterilized in an autoclave (121 ºC; 20 min), soaked with 5 mL of each extract and maintained in a germination chamber at 25 ºC and a 12-h light photoperiod, for seven days (Brasil 2009).

Daily germination monitoring was carried out from the day after sowing, with germination considered as seeds that presented a radicle protrusion (Ferreira & Borghethi 2004). The germination percentage, germination rate index (Maguire 1962) and mean germination time (Ferreira & Borghethi 2004) were calculated.

At the end of seven days, the seedlings were packed in paper bags and maintained in a forced circulation oven (65 ºC) until constant mass, to establish the biomass.

The results were submitted to a regression analysis at 1 % and 5 %, and the model was chosen according to the logic of the biological phenomenon, normality, regression significance and high coefficient of determination.

RESULTS AND DISCUSSION

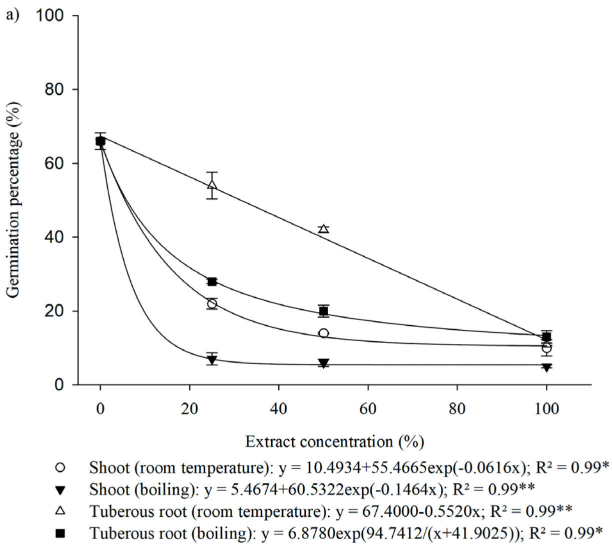
With increasing extracts concentrations, there was a reduction in germination percentages (Figure 1a). No interaction between factors was observed for germination percentage, although the extracts prepared with boiling water reduced the germination percentage more clearly than the extracts prepared with water at room temperature. The high temperature aqueous extract preparation condition may have resulted in a better extraction and yield of allelopathic substances present in *P. erosus*.

The shoot extract prepared by boiling, in addition to presenting a greater germination percentage inhibition, also reduced the germination rate and mean germination time to lower values than the control (Figures 1b and 1c).

The germination rate refers to the number of seeds germinated over time, while the mean germination time corresponds to the mean period required for all seeds to germinate (Ferreira & Borghethi 2004). *E. heterophylla* seeds presented delays at the beginning of the germination process, but with germinability synchrony, where all seeds germinated during the same time frame.

Thus, the cultivation of *P. erosus* may aid in the integrated management of weeds, since the observed germination delay, associated with synchrony, reduces the competition between species during the initial crop development (Bulegon et al. 2017).

The aqueous extracts prepared with boiling water, similarly to the results observed for *E. heterophylla*, reduced *B. pilosa* germination percentages more clearly than extracts prepared with water at room temperature, especially at the highest concentrations evaluated (Figure 2a).



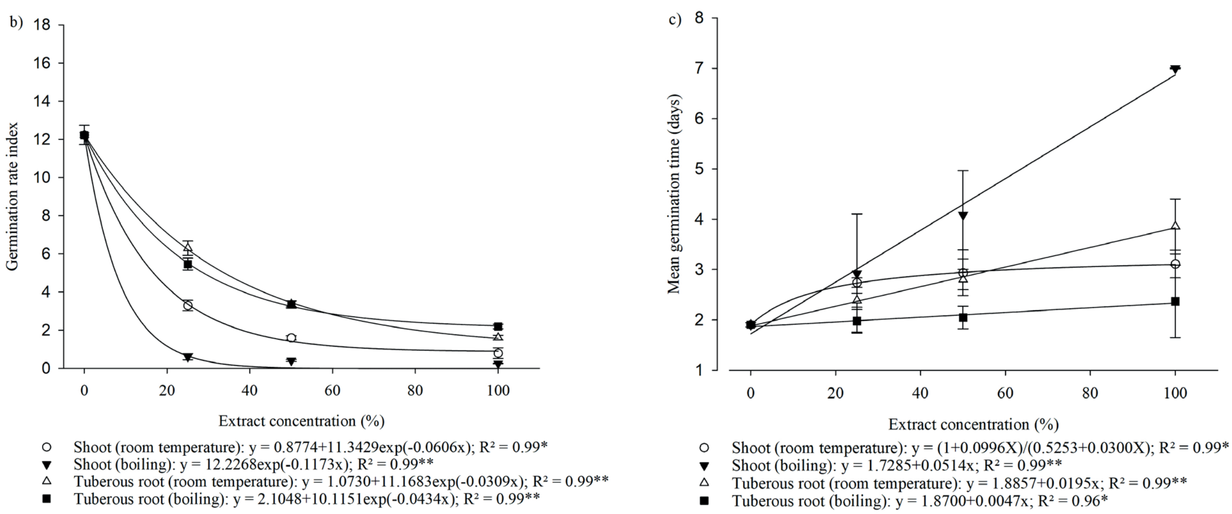


Figure 1. Germination percentage (a), germination rate index (b) and mean germination time (c) for *Euphorbia heterophyla*, after the application of aqueous extracts prepared with *Pachyrhizus erosus*. * and **: 5 % and 1 % of error probability, respectively.

Increasing the aqueous *P. erosus* extract concentrations led to greater decreases in the germination percentage, with interaction between all the factors evaluated. The shoot extract prepared with boiling water is noteworthy, leading to a decrease in germination of 89 % at 25 % of the extract concentration. At a 100 % extract concentration, germination was reduced in 92 % (Figure 2). Thus, it would take a four-fold higher amount of the aqueous shoot extract prepared with water at room temperature to achieve the same results as the aqueous shoot extract prepared with boiling water.

No triple interaction between the factors was observed for germination rate. However, an interaction between the preparation method and the concentration of the aqueous extracts was found. The highest decrease in this variable was observed when the aqueous shoot extract prepared with boiling water was used, which, even at 25 %, resulted in values close to zero (Figure 2b).

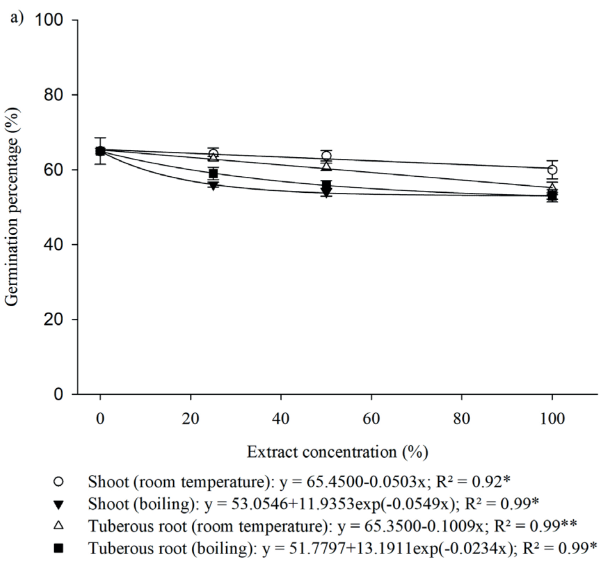
The decreases observed in the germination rate index are a result of the low germination of the seeds exposed to the aqueous extracts, similarly to the results reported by Alves et al. (2011).

For *B. pilosa*, the reduced number of germinated seeds resulted in a high mean germination time, which represents asynchronous germination in this species. Thus, there is a need for a longer period of time for all seeds to germinate. Asynchronous germination may be related to weed characteristics, where not all individuals germinate at the same time, aiming at survival.

All the extracts prepared with *P. erosus* caused decreases in the *E. heterophylla* and *B. pilosa* dry seedling biomass, with a triple interaction between the factors for both target species (Figure 3).

A 54 % decrease in dry biomass, if compared to the *E. heterophylla* controls, was observed when the aqueous tuberous root extract prepared with water at room temperature was applied (Figure 3a). However, this same extract caused only a 15 % decrease in the germination percentage (Figure 1a), indicating that *E. heterophylla* displays a greater sensitivity to this aqueous extract during its development, if compared to its germination period.

Regarding *B. pilosa*, on the other hand, the aqueous shoot extract prepared with boiling water



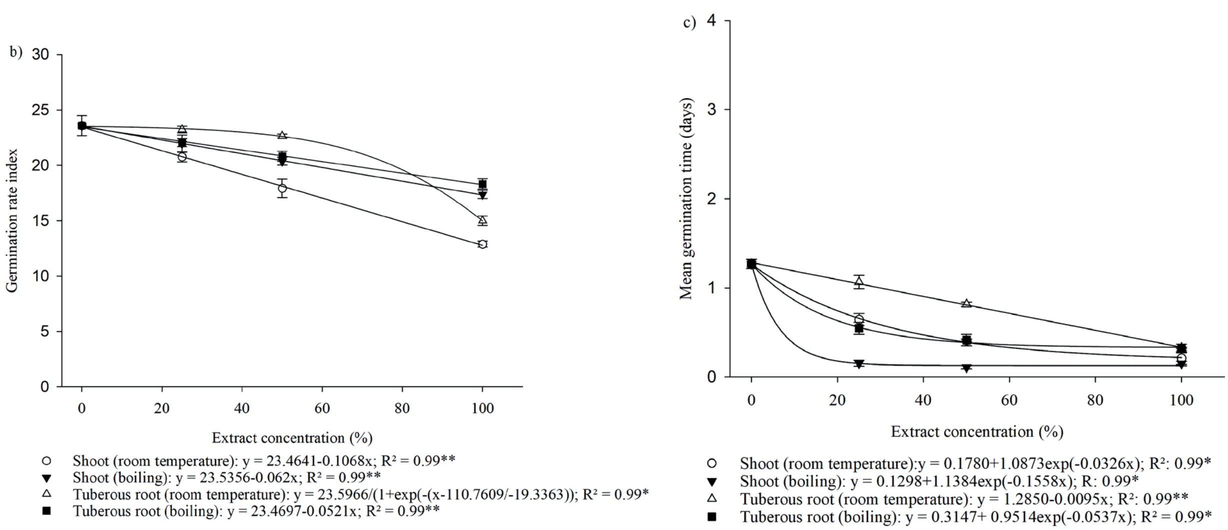


Figure 2. Germination percentage (a), germination rate index (b) and mean germination time (c) for *Euphorbia heterophyla*, after the application of aqueous extracts prepared with *Pachyrhizus erosus*. * and **: 5 % and 1 % of error probability, respectively.

reduced the dry biomass by 91 %, in relation to the control (Figure 3b), and was the most efficient extract, regarding the other evaluated parameters (Figure 2).

No studies applying *P. erosus* extracts in allelopathic tests with weeds are found in the literature. However, the results obtained herein were similar to those reported by other authors, such as Corsato et al. (2010), who observed that *B. pilosa* seed germination was totally inhibited when a 40 % aqueous sunflower leaves extract was applied.

The four classes of analyzed compounds were detected in *P. erosus* shoots (flavonoids, coumarins, saponins and tannins). However, tannins were not identified in the tuberous root aqueous extracts (Table 1).

The aqueous extracts prepared with boiling water were the most effective ones in reducing the weed germinability, probably due to the more efficient extraction of allelopathic substances. While evaluating different extraction methods, Leão et al. (2016) observed that water at 80 ºC allowed a more efficient extraction of polyphenols (flavonoids, tannins and coumarins), when compared to other methods evaluated.

Table 1. Qualitative phytochemical analysis of the shoot and tuberous root of *Pachyrhizus erosus* aqueous extracts.

Compound class	Shoot	Tuberous root
Flavonoids	+	+
Coumarins	+	+
Saponins	+	+
Tannins	+	-

(+) Presence; (-) absence.

Borella & Pastorini (2010) verified negative allelopathic effects of *Spondias tuberosa* fruits on *B. pilosa* seeds and attributed these results to the presence of flavonoids in the aqueous extracts, while Formagio et al. (2010) reported that flavonoids represent an important class of polyphenols with strong biological activity, such as plant hormone control action, allelopathic agents and enzymatic inhibitors.

In this context, Matsumoto et al. (2010) identified alkaloids, tannins and flavonoids in extracts with allelopathic activity, while Tur et al. (2010) identified saponins. These results allow us to propose that the identified compounds are related to the allelopathic effect observed on weeds.

Compounds present in plant material may also affect enzymes, such as endo-β-mannanase and β-mannosidase, which exhibit a high activity during the germination process (Suda et al. 2003). Furthermore, they may also inhibit the photosynthesis in young plants, as they are easily absorbed by hypocotyl and cotyledon tissues during early development (Dayan et al. 2009).

A relationship between secondary metabolism substances and chlorophyll content has been noted. Khaliq et al. (2012) observed that plants treated with wheat straw extracts presented proportionally decreased chlorophyll content, in relation to increased metabolic substances. Reducing chlorophyll content interferes with light energy uptake, and leads to inhibitory action on plant metabolism, such as cell

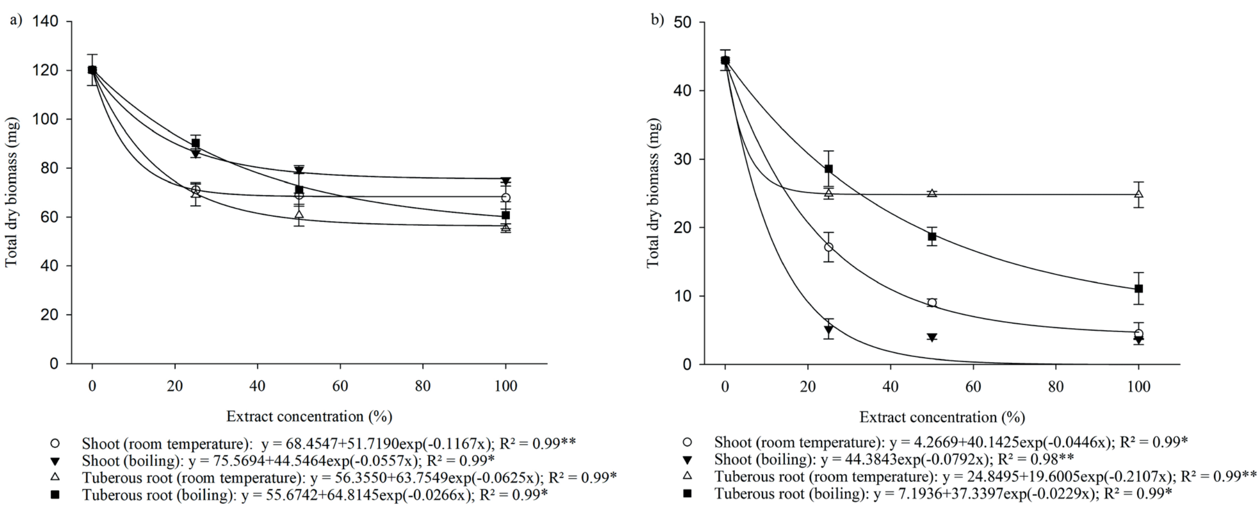


Figure 3. Total dry *Euphorbia heterophylla* (a) and *Bidens pilosa* (b) biomass, after the application of aqueous extracts prepared with *Pachyrhizus erosus*. * and **: 5 % and 1 % of error probability, respectively.

growth, multiplication and maintenance (Carmo et al. 2007).

Toxic compounds, such as flavonoids, coumarins, saponins and tannins, all identified herein, can induce the production of reactive oxygen species and lead to loss of selectivity of cell membranes (Tur et al. 2012). Faced with metabolic changes in the germinative process, seed vigor compromises, germination delays and alterations in germination rates and mean germination time occur, as observed by the results reported herein.

Magalhães et al. (1992) performed a chromatographic analysis of *P. erosus* seed extracts and observed the presence of flavonoids, the specific molecule pachirzine, also identified by Lautié et al. (2013), and rotenone, one of the best known specific molecules which, according to Ferreira et al. (2008), acts as a complex I inhibitor in the respiratory chain.

In spite of the reports of toxic substances in *P. erosus* organs, there is little evidence in the literature of its allelopathic activity on weeds, justifying further studies with this species, since it may aid in alternative control measures.

CONCLUSIONS

1. Aqueous *P. erosus* extracts exhibit an allelopathic effect on *E. heterophylla* and *B. pilosa* seeds, depending on the plant species organ, mode of preparation and concentration;
2. Greater decreases in *E. heterophylla* and *B. pilosa* germination percentages, germination rates and biomasses with increasing aqueous extract concentrations were observed, with the most evident results verified at higher extract concentrations;
3. Aqueous extracts prepared with boiling water lead to greater germination decreases than extracts prepared with water at room temperature, for both *E. heterophylla* and *B. pilosa*.

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