



## Phytotoxic potential of *Drimys brasiliensis* Miers for use in weed control

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**ABSTRACT.** This study aims to evaluate the phytotoxicity potential of leaf and root extracts of *Drimys brasiliensis* on the germination and seedling growth of *Panicum maximum* and *Euphorbia heterophylla* and its influence on metaxylem cell size in the seedling roots of the latter specie. The leaf and root extracts were fractionated by partition chromatography, and the hexane and ethyl acetate fractions obtained from each organ were evaluated at different concentrations for phytotoxic activity in several bioassays. In seedling growth tests, we compared the effects of these fractions with the herbicide oxyfluorfen. The hexane fraction of the root extracts showed a higher inhibitory potential on the germination and growth of weeds and reduced the average size of the metaxylem cells of *E. heterophylla* roots by more than 50%. The inhibitory effects of the root hexane fraction on seedling growth was similar to the herbicide, indicating that *D. brasiliensis* is a possible alternative form of control for the weed species examined.

**Keywords:** allelopathy, inhibition, germination, early growth.

### Potencial fitotóxico de *Drimys brasiliensis* Miers para o controle de plantas daninhas

**RESUMO.** O objetivo do estudo foi avaliar o potencial fitotóxico de frações do extrato de folhas e raízes de *Drimys brasiliensis* sobre a germinação e o crescimento de plântulas de *Panicum maximum* e *Euphorbia heterophylla*, e sua influência sobre as células do metaxilema da última espécie. Os extratos de folhas e raízes foram fracionados por cromatografia de partição e as frações hexânica e acetato de etila obtidas de cada órgão foram avaliadas nos bioensaios, em diferentes concentrações. No teste de crescimento, comparou-se o efeito das soluções das frações com o herbicida oxyfluorfen. A fração hexânica das raízes apresentou maior potencial para inibir a germinação e crescimento das espécies infestantes e ocasionou redução em mais de 50% no tamanho das células do metaxilema de *E. heterophylla*. As inibições no crescimento inicial, decorrentes da aplicação da fração hexânica das raízes, foram similares ao efeito produzido pelo herbicida comercial, indicando a eficiência de *D. brasiliensis* para o controle das espécies infestantes estudadas.

**Palavras-chave:** alelopatia, inibição, germinação, crescimento inicial.

### Introduction

Plants are capable of producing secondary metabolites that affect the germination and growth of other plants; this interaction has been defined as allelopathy (INDERJIT et al., 2011; WEIR et al., 2004). Allelopathic relationships may occur in a positive or negative manner through the production of chemical compounds known as allelochemicals. Allelochemicals are released into the environment by different mechanisms, such as volatilization, leaf leaching or root exudation (WEIR et al., 2004). The leaves and roots constitute the main source of allelochemicals (WU et al., 2009); however, due to the specific production of metabolites that are directly exuded into the environment, many compounds with relevant biological activities have

been isolated almost uniquely from the roots of these plants (OLIVEROS-BASTIDAS et al., 2009).

Weeds are adaptable to different habitats and often compete with crops by taking advantage of the favorable conditions that occur in agricultural systems. The indiscriminate application of synthetic herbicides has contributed to an increase in herbicide resistance in weeds, has led to a gradual degradation of soil quality and the surrounding environment and presents a human health hazard (VERDEGUER et al., 2011). Secondary metabolites derived from plants may serve as a promising environmentally friendly tool for weed management. Allelochemicals have low or no toxicity to animals and beneficial insects and possess an array of activities with varying and diverse sites of action. In addition, allelochemicals exhibit faster

degradation rates and contain fewer halogen substituents than synthetic herbicides (FAROOQ et al., 2011).

Many secondary metabolites derived from plants, such as drimanes sesquiterpenes, are present in species of the genus *Drimys* and exhibit a wide variety of biological activities, including leishmanicidal (CORRÊA et al., 2011), herbicidal and insecticidal activities (ZAPATA et al., 2009; 2011). In addition, secondary metabolites can induce cytotoxicity and affect plant-growth regulatory activities (JANSEN; GROOT, 2004). Thus, species of *Drimys* are promising in the search for phytochemicals capable of acting as natural herbicides. *Drimys brasiliensis* Miers, commonly called “casca d’anta”, belongs to the Winteraceae family and is found in the Atlantic Forest and gallery forests that extend into the Cerrado domain (EHRENDORFER et al., 1979). Previous studies have shown that it contains fungicidal (MALHEIROS et al., 2005) and anti-inflammatory properties (LAGO et al., 2010). However, few studies have examined its potential phytotoxicity, particularly with respect to its activity against agricultural weeds.

*Panicum maximum* Jacq. (guinea grass) is an alien grass known as one of the most important weeds in the sugarcane areas and planted forests of Brazil. Its interference with agricultural productivity is primarily due to its highly aggressive growth patterns, its intense production capacity and the longevity of its seeds (COSTA et al., 2002). Similarly, *Euphorbia heterophylla* L. (wild poinsettia) is a weed that negatively affects the productivity of agricultural systems, particularly soybean crops. Furthermore, it is considered difficult to control and shows resistance to several herbicide action mechanisms (TREZZI et al., 2009).

In this context, the aim of this study is to evaluate the phytotoxic effects of fractionated crude extracts of *D. brasiliensis* roots and leaves on the germination and early growth of *E. heterophylla* and *P. maximum*, as well as its influence on the metaxylem cell size of *E. heterophylla* roots.

## Material and methods

### Preparation of the chemical fractions

The leaves and roots of *D. brasiliensis* were collected in September 2011 from the Savanna area on the São Carlos Federal University (UFSCar) campus in São Carlos-SP (22° 2' S and 47° 52' W), Brazil. After collection, the leaves and roots were dried at 40°C for 72 hours and ground into a powder with an industrial mill.

For *D. brasiliensis* phytochemical extraction and the initial chromatography studies, 50 g of leaf and root powder, separately, were extracted with dichloromethane (DCM)/methanol (MeOH) (1:1) (5 x 200 mL). The resulting extracts were vacuum filtered, pooled and concentrated in a rotary evaporator under reduced pressure. Each concentrated crude extract was suspended in 95% MeOH (200 mL) and partitioned with n-hexane (3 x 200 mL), resulting in both methanol and hexane (2.38 g) fractions. The methanol fraction was concentrated, resuspended in distilled water (200 mL) and partitioned with ethyl acetate (3 x 200 mL), yielding two fractions: an ethyl acetate (AcOEt) (2.88 g) fraction and an aqueous (6.32 g) fraction (RANGEL et al., 2001). At the end, the aqueous fractions of both organs were discarded, and the hexane and ethyl acetate fractions were evaporated, weighed and subjected to phytotoxicity bioassays.

In each bioassay, 40 mg of each leaf or root extract (hexane and AcOEt fractions) were solubilized in 40 mL of buffer solution (10 mM 2-[N-morpholino] ethanesulfonic acid (MES) and 1 M NaOH, pH 6) and DMSO (dimethyl sulfoxide, 5  $\mu\text{L mL}^{-1}$ ), resulting in an initial concentration of 1 mg of extract  $\text{mL}^{-1}$ . From this solution, dilutions of 0.5, 0.25 and 0.125 mg  $\text{mL}^{-1}$  were prepared. For each bioassay, a negative control with only buffer solution and DMSO (5  $\mu\text{L mL}^{-1}$ ) was also assessed (MACÍAS et al., 2010).

### Germination bioassay

The hexane and AcOEt fractions of *D. brasiliensis* leaf and root extract were applied to *E. heterophylla* and *P. maximum* seeds. Bioassays were conducted in Petri dishes (9 cm in diameter) containing two sheets of filter paper moistened with 5 mL of either the sample fractions or the negative control solution.

The experimental design was completely randomized and contained four replicates of 25 seeds for each bioassay. The experiment was conducted in a germination chamber at 25°C, with a photoperiod of 12 hours for *E. heterophylla* (INOUE et al., 2010) and alternating temperatures of 20-30°C and a photoperiod of 8-16h for *P. maximum* (TOMAZ et al., 2010). The germination criterion was based on embryo protrusion, which was evaluated every 24 hours until germination was stabilized. The germinability, mean germination time and synchrony were calculated as described by Ranal and Santana (2006).

### Early growth bioassay

For the early growth analysis of *E. heterophylla* and *P. maximum* seedlings, the seeds were previously germinated in distilled water under the same conditions mentioned for the germination bioassay. Only seedlings with roots of 2 mm in length were selected and transferred to transparent plastic boxes (13 x 8 x 3 cm) containing filter paper moistened with 5 mL of the negative control solution, leaf or root fractions, or herbicide. For this test, a bioassay was performed with the commercial herbicide oxyfluorfen (240 g i.a. L<sup>-1</sup>) at the same concentrations as the fractionated extracts.

The boxes were maintained in a germination chamber under the same conditions of light and temperature adopted for the germination test. The experimental design was completely randomized and contained four replicates of 10 seedlings. After seven days, the shoot and primary root lengths of the seedlings were measured with a caliper. The presence or absence of any anomalies was noted, and the seedlings were classified as normal or abnormal, according to Brasil (2009).

### Examination of metaxylem cells

*E. heterophylla* seedlings were grown in control or *D. brasiliensis* leaf or root extract fractions under the same conditions adopted for the growth bioassay. After five days, the primary root segments of the seedlings were removed and immersed in 70% alcohol (GATTI et al., 2010). Primary root segments were subjected to a modified Fuchs staining method (KRAUS; ARDUIN, 1997). Briefly, the roots were immersed in 70% alcohol for five days and placed in a solution of 25% NaOH at 60°C for 48 hours to clarify the material.

The root segments were subsequently immersed in safranin (C<sub>20</sub>H<sub>19</sub>N<sub>4</sub>C<sub>1</sub>) and caustic soda (10% NaOH) for 24 hours at 60°C. After staining, the segments were mounted on glass slides with Apathy's syrup (KRAUS; ARDUIN, 1997) and observed with an optical microscope (Olympus-BX41) coupled to a camera (Sony CCD-IRIS) at 20X magnification. Four primary roots of *E. heterophylla* seedlings grown in different concentrations of the fractions and control solutions were assessed. Half of the length of each root from the central region upward was photographed. From each photograph, 10 central cells of the metaxylem were measured with the aid of Image Pro Plus 5.0 software (GATTI et al., 2010).

### Statistical Analysis

The data were tested for normality (Shapiro-Wilk) and homogeneity (Levene). Once these two

assumptions were met, an analysis of variance (ANOVA) was performed followed by Tukey's or Dunnett tests at a significance level of 0.05. A lack of normality or homogeneity (or both) required the use of a nonparametric Kruskal-Wallis test to obtain pairwise comparisons at a significance level of 0.01. For the germination and growth bioassays, the linear or quadratic regression models were adjusted when the ANOVA *F* statistic was significant. The goodness of fit of the models was tested at a level of significance of 0.05 and evaluated by its coefficient of determination (R<sup>2</sup>). For the variables that showed no significant differences between treatments, the results were presented in tables.

The early growth data were submitted to conjoint analysis because the ratio between the larger and smaller residual mean square was less than 7 (PIMENTEL-GOMES, 1990).

## Results and discussion

### Germination and growth bioassays

The hexane and AcOEt root fractions of *D. brasiliensis* strongly inhibited the germination process and early growth of *P. maximum*. The AcOEt fraction promoted a linear decrease in germination and synchrony (17% and 0.157 for each additional 0.01 mg mL<sup>-1</sup> of solution applied, respectively), and mean germination time increased in a linear manner (1.57 days for each additional 0.01 mg mL<sup>-1</sup> of solution applied). In the presence of the hexane fraction, a linear increase in mean germination time of 2.56 days for each additional 0.01 mg mL<sup>-1</sup> of solution was observed, and the minimum germinability (13.79%) and synchrony (0.06) values were estimated at concentrations of 0.75 and 0.69 mg mL<sup>-1</sup>, respectively (Figure 1A to C).

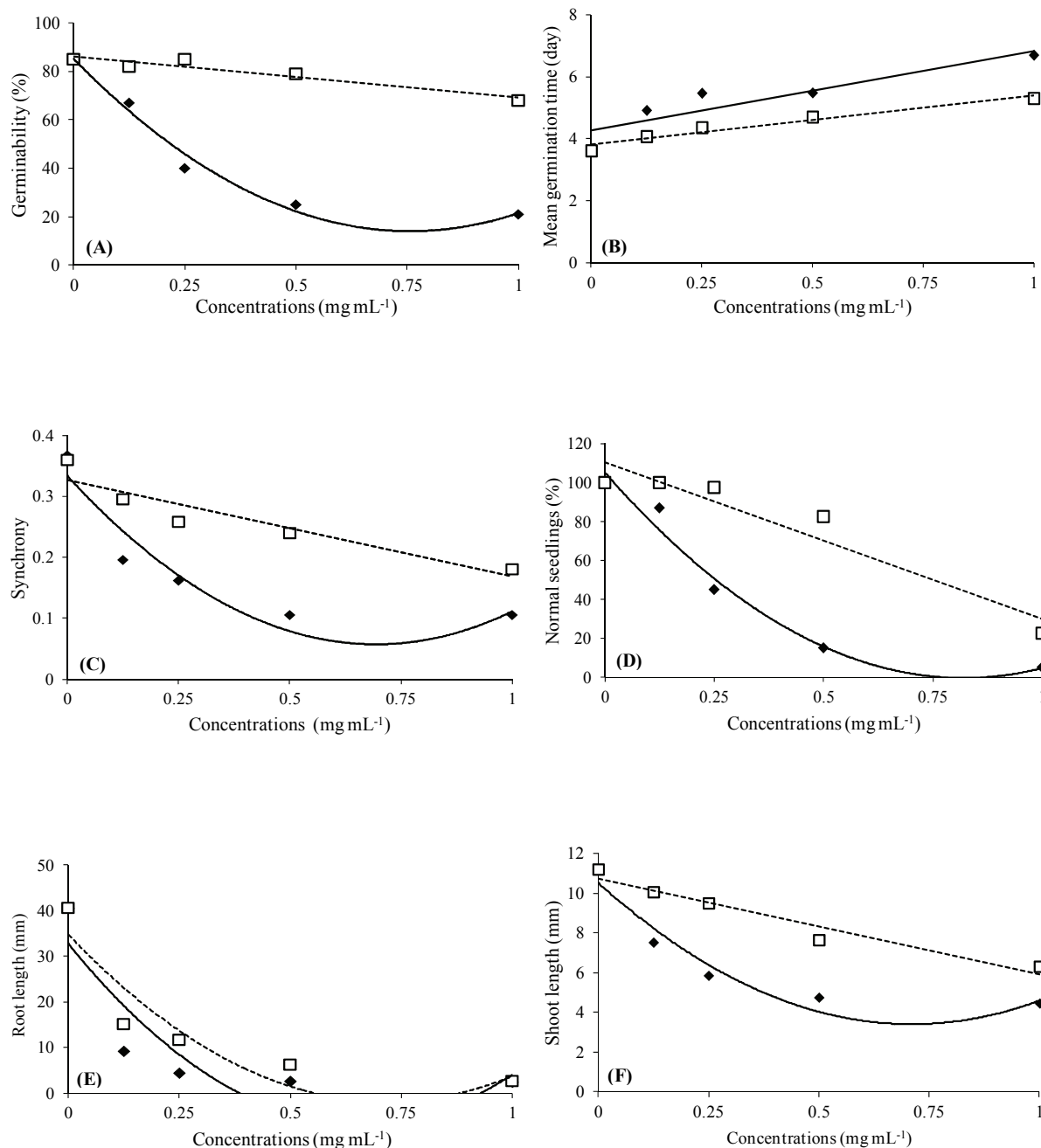
The root extract fractions effectively inhibited the growth and changed the morphology of *P. maximum* seedlings. The percentage of normal seedlings decreased linearly at a rate of 80.5% for each 0.01 mg mL<sup>-1</sup> of AcOEt fraction solution added. The hexane fraction reduced the normal seedling percentage to zero at an estimated concentration of 0.81 mg mL<sup>-1</sup> (Figure 1D).

The root growth of seedlings in the presence of the AcOEt and hexane fractions was reduced to null at estimated concentrations of 0.65 and 0.71 mg mL<sup>-1</sup>, respectively. A linear decrease in shoot growth of 4.80 mm for each additional 0.01 mg mL<sup>-1</sup> of AcOEt fraction applied was observed. The minimum shoot length (3.40 mm) was observed in the hexane

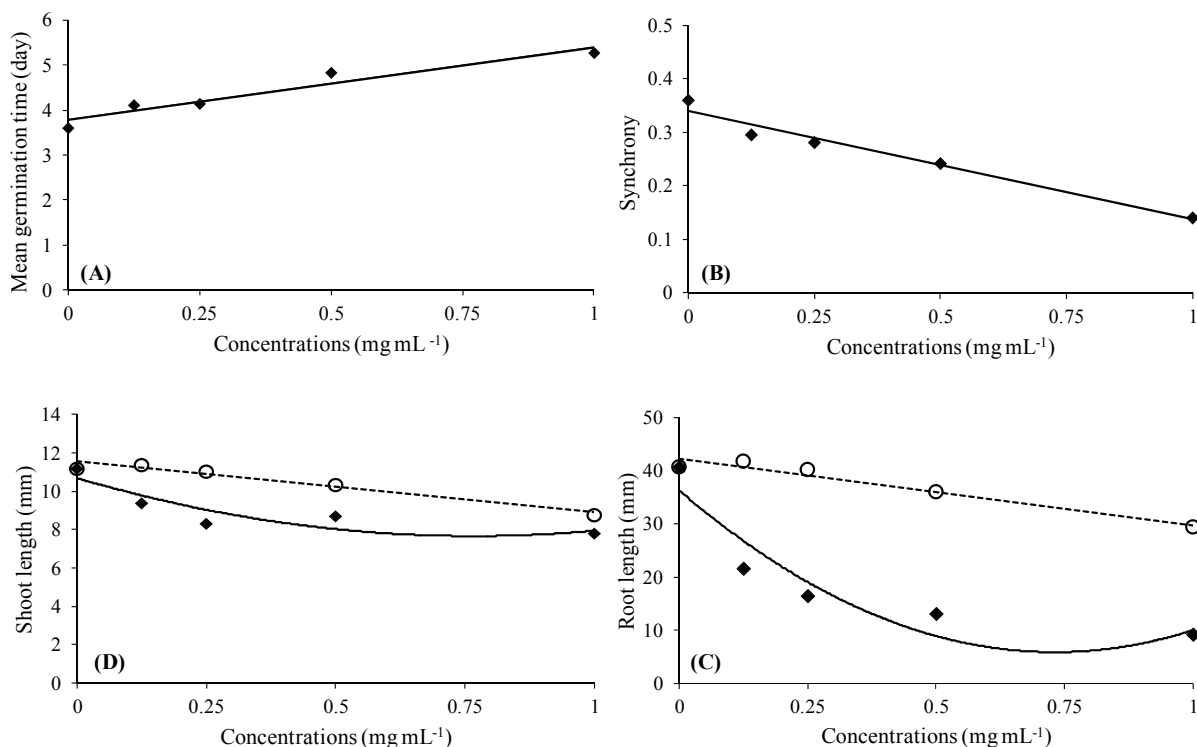
fraction treated group at a concentration of  $0.71 \text{ mg mL}^{-1}$  (Figure 1E and F).

Treatment with hexane and AcOEt fractions of *D. brasiliensis* leaf crude extract revealed a significant inhibition of some of the *P. maximum* germination and growth parameters evaluated. The mean time and synchrony of seed germination after hexane

fraction treatment displayed a linear increase of 1.60 days and a linear decrease of 0.2017 for each additional  $0.01 \text{ mg mL}^{-1}$  of solution applied, respectively (Figure 2A and B). All other variables analyzed showed no significant differences among the concentrations when treated with either fraction (Table 1).



**Figure 1.** Germination and early growth of *P. maximum* treated with different concentrations of the AcOEt ( $\square$ ; --- $\gamma$ ) and hexane ( $\blacklozenge$ ; — $\gamma$ ) fractions obtained from *D. brasiliensis* roots. Equations were obtained by regression analysis: (A)  $\gamma = 86.17 - 17x$ ,  $R^2 = 0.90$ ;  $\gamma = 85.09 - 0.188x + 0.0001x^2$ ,  $R^2 = 0.99$ ; (B)  $\gamma = 3.80 + 1.575x$ ,  $R^2 = 0.94$ ;  $\gamma = 4.26 + 2.56x$ ,  $R^2 = 0.82$ ; (C)  $\gamma = 0.325 - 0.157x$ ,  $R^2 = 0.87$ ;  $\gamma = 0.3336 - 0.7966x + 0.574x^2$ ,  $R^2 = 0.91$ ; (D)  $\gamma = 110.599 - 80.5x$ ,  $R^2 = 0.91$ ;  $\gamma = 105.35 - 257.85x - 157.14x^2$ ,  $R^2 = 0.97$ ; (E)  $\gamma = 34.81 - 102.26x + 71.20x^2$ ,  $R^2 = 0.85$ ;  $\gamma = 32.79 - 120.17x + 91.37x^2$ ,  $R^2 = 0.78$ ; (F)  $\gamma = 10.74 - 4.805x$ ,  $R^2 = 0.94$ ;  $\gamma = 10.52 - 20.04x + 14.10x^2$ ,  $R^2 = 0.94$ .



**Figure 2.** Germination and early growth of *P. maximum* treated with different concentrations of the AcOEt (○; ---) and hexane (◆; —) fractions obtained from *D. brasiliensis* leaves. Equations were obtained by regression analysis: (A)  $y = 3.788 - 1.604x$ ,  $R^2 = 0.92$ ; (B)  $y = 0.3394 - 0.2017x$ ,  $R^2 = 0.96$ ; (C)  $y = 42.351 - 12.57x$ ,  $R^2 = 0.95$ ;  $y = 36.34 - 83.45x + 57.11x^2$ ,  $R^2 = 0.88$ ; (D)  $y = 11.544 - 2.636x$ ,  $R^2 = 0.95$ ;  $y = 10.685 - 7.8851x + 5.146x^2$ ,  $R^2 = 0.79$ .

**Table 1.** Seed germination and seedling morphology of *P. maximum* treated with different concentrations of hexane or AcOEt fractions from *D. brasiliensis* leaf extract.

Variables (units)	Concentrations (mg mL <sup>-1</sup> )					Statistics	
	0	0.125	0.25	0.5	1	F (P)	H (P)
Hexane fraction of leaves							
G (%)	85 ± 7.5 a	78 ± 9.5 a	78 ± 9.5 a	80 ± 8.64 a	79 ± 4.6 a	0.487 (0.745)	11.39 (0.062)
NS (%)	100 ± 0 a	97 ± 5 a	80 ± 7 a	77 ± 11 a	75 ± 10 a		
AcOEt fraction of leaves							
G (%)	85 ± 7.57 a	81 ± 6.83 a	84 ± 4.61 a	84 ± 3.26 a	79 ± 8.24 a	0.618 (0.657)	3.87 (0.423)
MGT (d)	3.6 ± 0.18 a	3.5 ± 0.25 a	3.72 ± 0.34 a	3.87 ± 0.37 a	3.88 ± 0.24 a	0.931 (0.472)	
Z	0.36 ± 0.02 a	0.43 ± 0.09 a	0.34 ± 0.08 a	0.34 ± 0.31 a	0.31 ± 0.08 a	0.897 (0.490)	
NS (%)	100 ± 0 a	100 ± 0 a	97.5 ± 5 a	95 ± 5.7 a	92.5 ± 7 a		

Means followed by the same letter in the line do not differ by tukey's test at a 0.05 level of significance; G: germinability; MGT: mean germination time; Z: synchrony; NS: normal seedlings; F: test statistic F - Values indicated no significant differences among the concentrations (ANOVA;  $p > 0.05$ ); H: statistic of the Kruskal-Wallis test - Values indicated no significant differences among the concentrations ( $p > 0.05$ ); p: probability.

The growth of *P. maximum* was sensitive to the leaf extract fractions. Upon treatment with the AcOEt fraction, linear decreases of 12.57 and 2.63 mm were observed on root and shoot growth, respectively, for each additional 0.01 mg mL<sup>-1</sup> of solution applied. Upon treatment with the hexane fraction, the minimum root length (5.86 mm) was observed at a concentration of 0.73 mg mL<sup>-1</sup>, whereas the minimum shoot length (7.65 mm) was observed at an estimated concentration of 0.76 mg mL<sup>-1</sup> (Figure 2D and C). The percentage of normal seedlings was not significantly affected by the leaf extract fractions (Table 1).

Root extract fractions from *D. brasiliensis* did not significantly affect most of the germination variables of *E. heterophylla* (Table 2); however, a minimum germination value (32.6%) upon treatment with the hexane fraction was significantly affected at an estimated concentration of 0.64 mg mL<sup>-1</sup> (Figure 3A).

Despite the results observed in the germination bioassay, the root extract fractions exerted strong phytotoxic effects on the morphology and growth of *E. heterophylla* seedlings. The minimum root length values (0 and 2.01 mm) were observed at estimated

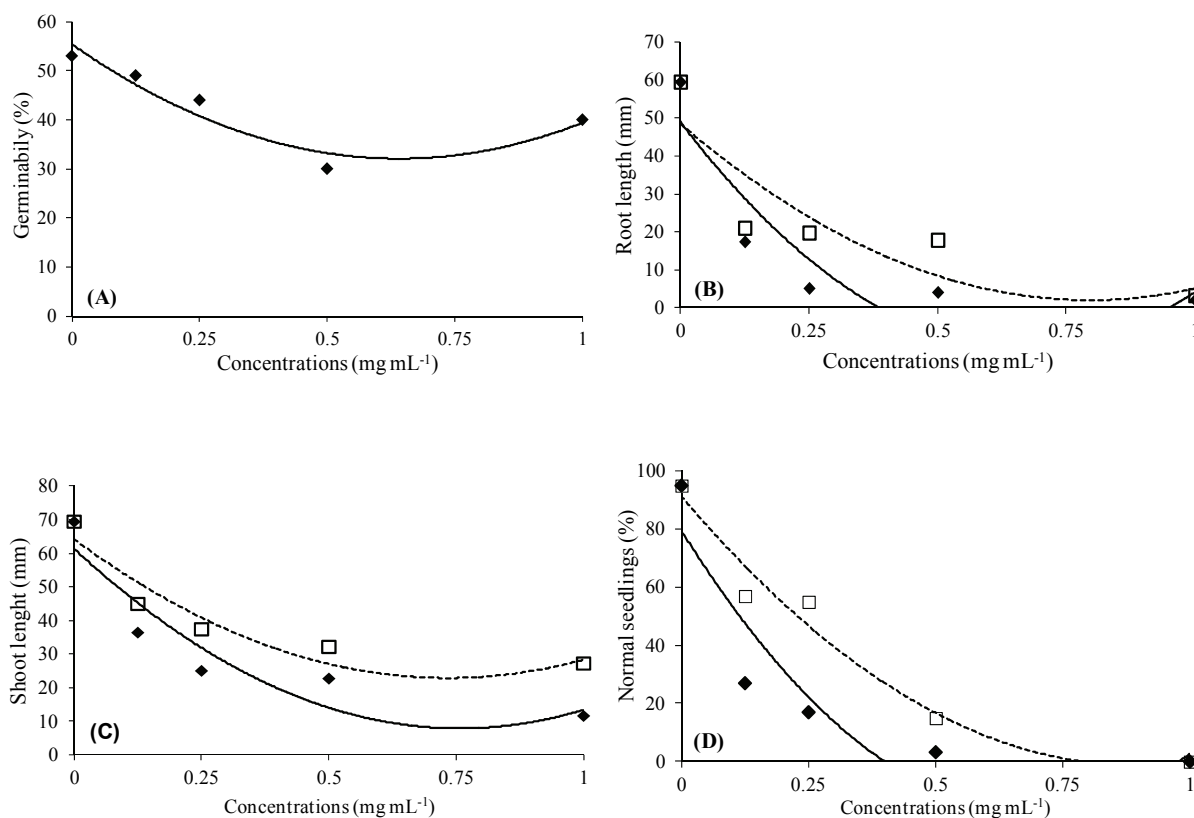
concentrations of 0.66 and 0.79 mg mL<sup>-1</sup> for hexane and AcOEt fractions, respectively. The minimum shoot length of seedlings treated with hexane (7.83 mm) or AcOEt (23.39 mm) fractions were observed at estimated concentrations of 0.73

and 0.75 mg mL<sup>-1</sup>, respectively. Normal seedling percentages ranged from 95% (control) to 0% at estimated concentrations of 0.68 and 0.89 mg mL<sup>-1</sup> for hexane and AcOEt fractions, respectively (Figure 3B to D).

**Table 2.** Seed germination and seedling morphology of *E. heterophylla* upon treatment with different concentrations of *D. brasiliensis* root and leaf extract fractions.

Variables (units)	Concentrations (mg mL <sup>-1</sup> )					Statistics	
	0	0.125	0.25	0.5	1	F (P)	H (P)
Hexane fraction of roots							
MGT (d)	1.54 ± 0.14 a	1.65 ± 0.12 a	1.75 ± 0.25 a	1.81 ± 0.16 a	1.96 ± 0.16 a	2.051 (0.138)	
Z	0.46 ± 0.12 a	0.40 ± 0.03 a	0.40 ± 0.13 a	0.42 ± 0.18 a	0.37 ± 0.13 a	0.246 (0.907)	
AcOEt fraction of roots							
G (%)	53 ± 7.57 a	44 ± 8.64 a	50 ± 9.52 a	49 ± 3.83 a	43 ± 5.03 a	1.348 (0.298)	
MGT (d)	1.54 ± 0.28 a	1.75 ± 0.11 ab	1.83 ± 0.15 ab	1.89 ± 0.18 ab	2.02 ± 0.15 b	3.669 (0.028)	
Z	0.46 ± 0.12 a	0.33 ± 0.05 a	0.34 ± 0.05 a	0.37 ± 0.33 a	0.30 ± 0.08 a	2.492 (0.070)	
Hexane fraction of leaves							
G (%)	53 ± 7.57 a	52 ± 12.64 a	44 ± 6.53 a	56 ± 9.79 a	47 ± 13.21 a	0.878 (0.500)	
MGT (d)	1.54 ± 0.28 a	1.81 ± 0.16 a	1.77 ± 0.03 a	1.88 ± 0.19 a	1.93 ± 0.24 a	2.214 (0.117)	
Z	0.46 ± 0.12 a	0.34 ± 0.04 a	0.35 ± 0.03 a	0.37 ± 0.08 a	0.32 ± 0.06 a	1.976 (0.150)	
AcOEt fraction of leaves							
G (%)	53 ± 7.57 a	49 ± 8.24 a	51 ± 16.97 a	40 ± 14.69 a	41 ± 12.80 a		3.731 (0.444)
MGT(d)	1.51 ± 0.28 a	1.66 ± 0.21 a	1.59 ± 0.02 a	1.64 ± 0.13 a	1.65 ± 0.03 a		1.758 (0.780)
Z	0.46 ± 0.12 a	0.44 ± 0.07 a	0.44 ± 0.05 a	0.36 ± 0.02 a	0.47 ± 0.06 a	1.260 (0.329)	
NS (%)	95 ± 5.77 a	77.5 ± 5 a	65 ± 10 a	65 ± 8 a	72.2 ± 7 a	2.827 (0.062)	

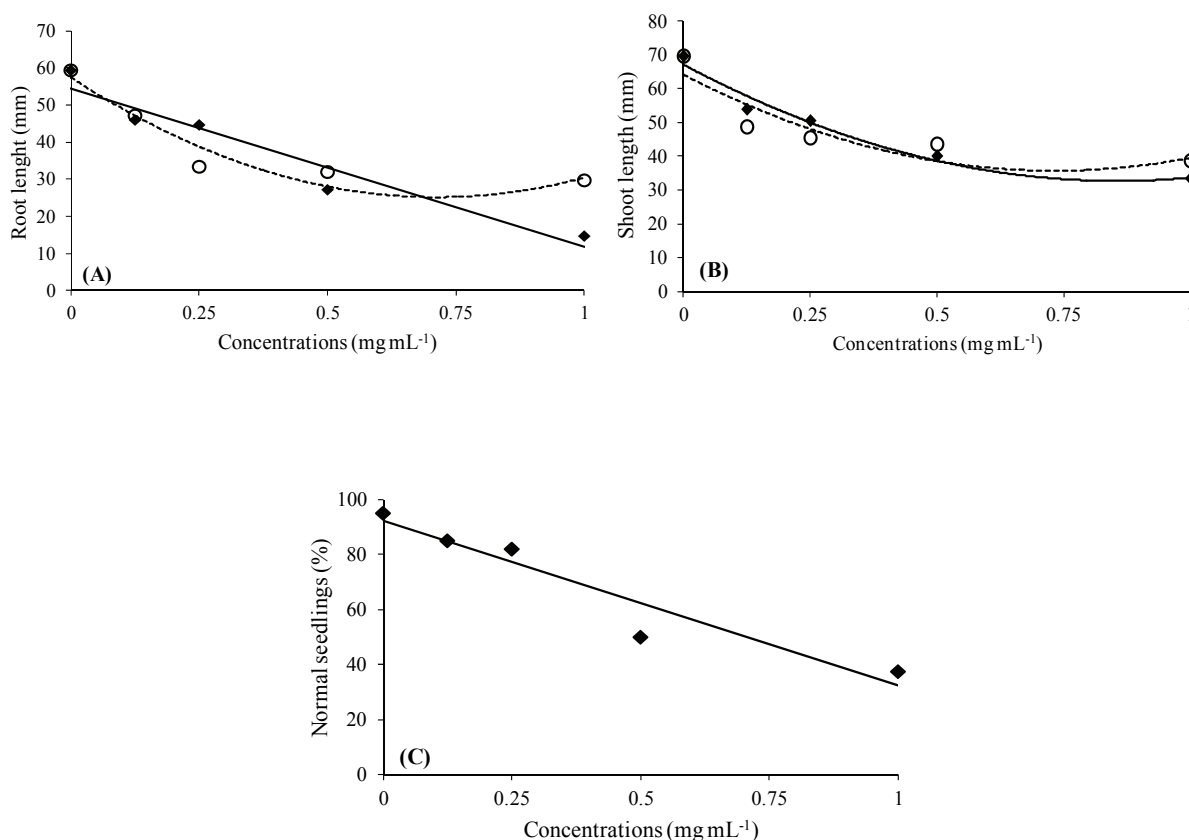
Means followed by the same letter in the line do not differ by tukey's test at a 0.05 level of significance; G: germinability; MGT: mean germination time; Z: synchrony; NS: normal seedlings; F: test statistic F – Values in bold indicate significant difference among the concentrations (ANOVA; p < 0.05); H: statistic of the Kruskal-Wallis test; P: probability.



**Figure 3.** Germination and early growth of *E. heterophylla* treated with different concentrations of AcOEt (□; ---) and hexane (♦; —) fractions obtained from *D. brasiliensis* root extracts. Equations were obtained by regression analysis: (A)  $y = 55.4 - 72.74x + 56.77x^2$ ,  $R^2 = 0.90$ ; (B)  $y = 48.55 - 116.95x + 73.488x^2$ ,  $R^2 = 0.75$ ;  $y = 49.002 - 177.97x + 133.08x^2$ ,  $R^2 = 0.81$  (C)  $y = 64.195 - 112.75x + 76.874x^2$ ,  $R^2 = 0.90$ ;  $y = 61.35 - 141.39x + 93.378x^2$ ,  $R^2 = 0.80$ ; (D)  $y = 91.35 - 0.207x + 0.0001x^2$ ,  $R^2 = 0.96$ ;  $y = 79.65 - 0.2823x + 0.0002x^2$ ,  $R^2 = 0.85$ .

The germination variables examined for *E. heterophylla* seeds treated with the *D. brasiliensis* leaf extract fractions were not significantly different among all concentrations (Table 2); thus, the leaf extract exerted no phytotoxic effects on the germination of this species. In contrast, the leaf extract fractions inhibited the growth of *E. heterophylla* seedlings. The minimum shoot length (32.8 mm) was observed at a concentration of 0.87 mg mL<sup>-1</sup> after application of the hexane fraction. In addition, a linear reduction in root length (48.26 mm) was observed for each additional 0.01 mg mL<sup>-1</sup> of solution applied. The seedlings treated with the AcOEt fraction displayed lower shoot (35.7 mm) and root (25.15 mm) lengths at estimated concentrations of 0.73 and 0.71 mg mL<sup>-1</sup>, respectively. Seedling morphology was altered upon treatment with the hexane fraction, displaying a linear reduction of 59.9% for each additional 0.01 mg mL<sup>-1</sup> of solution added (Figure 4A to C).

The *D. brasiliensis* root and leaf extract fractions exerted phytotoxic effects on *P. maximum* and *E. heterophylla* as measured by several characteristics: inhibition was positively associated with increased concentrations of extract fractions. The allelopathic effect depended upon the extract concentration, target species, and the plant tissue from which the allelochemicals were extracted (GNIAZDOWSKA; BOGATEK, 2005; HAO et al., 2007). The effective concentrations of plant extracts on weed control were previously reported to range from 0.1 to 100 mg mL<sup>-1</sup> (GRISI et al., 2012; TEERARAK et al., 2012; ZAPATA et al., 2011). For the characteristics evaluated in both weed species upon treatment with *D. brasiliensis* extract fractions, the greatest inhibitory effects occurred at concentrations estimated at 1 mg mL<sup>-1</sup>. Therefore, the effective concentrations evaluated in this study could be considered low, indicating the potential of *D. brasiliensis* to act as a donor species for the production of compounds with high phytotoxic potential.



**Figure 4.** Early growth of *E. heterophylla* treated with different concentrations of AcOEt (□; ---) and hexane (◆; —) fractions of *D. brasiliensis* leaf extract. Equations were obtained by regression analysis: (A)  $y = 57.46 - 90.68x + 63.63x^2$ ,  $R^2 = 0.92$ ;  $y = 54.467 - 42.826x$ ,  $R^2 = 0.93$ ; (B)  $y = 64.157 - 77.79x + 53.165x^2$ ,  $R^2 = 0.81$ ;  $y = 66.937 - 77.826x + 44.59x^2$ ,  $R^2 = 0.96$  (C)  $y = 92.363 - 59.9x$ ,  $R^2 = 0.91$ .



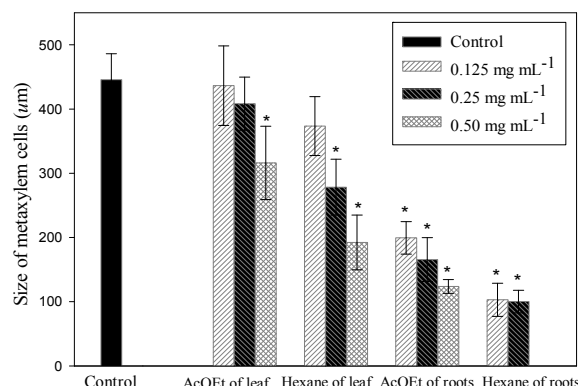
The phytotoxic activity of *D. brasiliensis* root and leaf extract fractions was stronger during early growth than during the germination phases in both weed species. In general, species with small seeds are more sensitive to allelochemicals than species with large seeds (BURGOS; TALBERT, 2000). Weeds with large seeds tend to be less sensitive to allelochemicals such as sorgoleone because these plants can minimize the phytotoxic effects by decreasing its absorption of the allelochemical, by translocation or by increasing the metabolic degradation rate of the phytotoxins (DAYAN, 2006). In this study, the germination of *E. heterophylla* was less sensitive to treatment with the *D. brasiliensis* fractions than *P. maximum*. Seeds of *E. heterophylla* are larger than the *P. maximum*; therefore, we infer that the seeds of this species are more tolerant to allelochemicals. However, the seed size does not completely explain the tolerance of some species to these compounds. As with commercial herbicides, the ability of a species to detoxify toxic compounds may contribute to tolerance (BURGOS et al., 2004).

The *D. brasiliensis* fractions inhibited the seedling growth of both weed species. In general, the root was the most sensitive organ, as previously reported in other studies (GATTI et al., 2010; GRISI et al., 2012; INOUE et al., 2010; SOUZA FILHO et al., 2010; TEERARAK et al., 2012). Souza Filho and Duarte (2007) have suggested that inhibition of root development is one of the main factors that indicate sensitivity to allelochemicals in a manner that is independent of the receiver species or the concentration. This inhibition is particularly important during early seedling development, which is characterized by high metabolism and environmental stress sensitivity (CRUZ-ORTEGA et al., 1998). Changes in the growth and development of seedling roots in response to allelochemicals can be explained by a reduction in the number of mitotic cells, inhibition of meristematic cell proliferation, inhibition of the cell cycle, disintegration of the root cap, increased diameter of the vascular cylinder and earlier lignification of the metaxylem (SANTOS et al., 2008; SOLTYS et al., 2011).

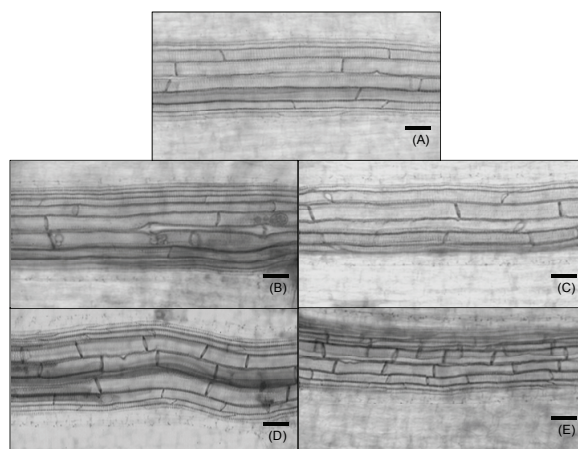
#### Examination of metaxylem cells in *E. heterophylla* seedlings

The anatomical study of *E. heterophylla* roots allowed us to better visualize the phytotoxic effects of the extracts at the cellular level. The roots grown in the presence of *D. brasiliensis* root extract fractions displayed a more significant reduction in the average size of the metaxylem cells compared to those

grown in the presence of leaf extract fractions and the control solution (Figures 5 and 6).



**Figure 5.** Average sizes of *E. heterophylla* root metaxylem cells in the presence of different concentrations of AcOEt and hexane fractions obtained from *D. brasiliensis* leaf and roots extracts compared to the control. (\*)Average differs significantly from control by the Dunnett test, at a level of significance of 0.05.



**Figure 6.** Photomicrographs of root metaxylem cells of *E. heterophylla* grown in control (A), AcOEt fraction of leaf extract (B), hexane fraction of leaf extract (C), AcOEt fraction of root extract (D) and hexane fraction of root extract (E) of *D. brasiliensis*, at a concentration of 0.25 mg mL<sup>-1</sup>. Scale = 50 µm.

The average size of the control seedling metaxylem cells was 445.64 µm. Seedlings grown in the presence of root extracts had a statistically smaller average metaxylem cell size than seedlings in the control group (more than 50%). The smallest average cell size (100.20 µm) was observed in seedlings treated with the hexane fraction of the root extract at a concentration of 0.25 mg mL<sup>-1</sup>. Values for the hexane fraction root extract treated seedlings could not be obtained for the 0.5 mg mL<sup>-1</sup> concentration, due to an absence of root growth and prevention of metaxylem formation (Figure 5).

The hexane fraction from *D. brasiliensis* root extract inhibited root growth of seedlings of



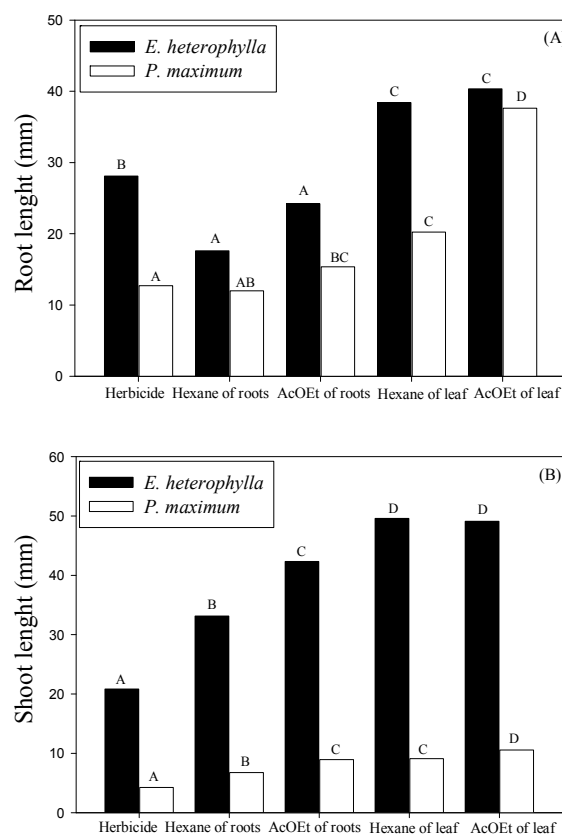
*E. heterophylla* at a higher level, suggesting that this inhibitory effect may be associated with a decrease in metaxylem cell elongation. This result is in agreement with the results presented by Gatti et al., (2010). The authors suggest that allelochemicals interfere with cell growth and that this reduction in cell size may be associated with changes in the concentration of hormones, such as auxins. Auxin is the major phytohormone controlling cell division, growth and differentiation (PERROT-RECHENMANN, 2010) and has a profound influence on root morphology and growth. Auxin is also thought to control the differentiation and growth of the primary vascular root tissues, the protoxylem and the metaxylem (ALONI et al., 2006). Thus, allelochemicals may interfere with the action of auxins, resulting in changes in cell growth.

#### Conjoint analyses for growth bioassay

The conjoint analysis allows for the comparison of the *D. brasiliensis* fractions data with the results obtained using the commercial herbicide. The AcOEt and hexane root extract fractions equally inhibited *E. heterophylla* root growth and displayed higher inhibitory activity than the commercial herbicide or leaf extract fractions. In addition, both root fractions displayed similar inhibitory effects on the root growth of *P. maximum*. However, the hexane fraction was not significantly different from the commercial herbicide, demonstrating its ability to control the growth of this weed (Figure 7A).

The commercial herbicide exerted greater phytotoxic effects on the shoot growth of weed species compared to the chemical fractions; however, the hexane fraction from root extracts exhibited the highest inhibitory potential for both species (Figure 7B). Thus, crude *D. brasiliensis* root extract fractions, particularly the hexane fraction, showed higher phytotoxicity towards the growth of target species than extracts obtained from the leaves. Previous reports have suggested that toxicity levels vary depending on the plant organ used during the extraction. The roots are the major source of phytotoxins in *Bauhinia guianensis* and *Parkia pendula* species (MOURÃO-JUNIOR; SOUZA FILHO, 2010). However, *Eragrostis plana* (FAVARETTO et al., 2011) and *Eucalyptus sp.* (ZHANG et al., 2010) exhibited greater phytotoxic activity from leaf extracts. The spectrum of phytotoxic effects observed after treatment with leaf and root extracts may be attributable to differences in the concentrations of diffusible allelochemicals, varied chemical composition, or differentiated alkalinity (DORNING; CIPOLLINI, 2006). Many compounds are synthesized in the roots and are

exuded directly into the environment without being detected in the aerial parts of the plant. Because of this specificity of the location of allelochemical synthesis, many compounds with promising biological activities have been isolated almost uniquely in roots (OLIVEROS-BASTIDAS et al., 2009).



**Figure 7.** Root (A) and shoot lengths (B) of *E. heterophylla* and *P. maximum* seedlings treated with hexane and AcOEt fractions obtained from *D. brasiliensis* root and leaf extracts or a commercial herbicide. Means followed by the same capital letters within each species do not differ by Tukey's test at a significance level of 0.05.

The conjoint analysis and the anatomical results revealed variations in the phytotoxic activity among *D. brasiliensis* fractions related to the polarity of the solvents used in the extraction. For both organs, the fractions obtained with the solvent n-hexane had higher levels of inhibitory activity, which suggests that substances with allelopathic potential present in *D. brasiliensis* are nonpolar. Classes of compounds with low polarity that originated from the extraction with hexane include the monoterpenes, oxygenated monoterpenes and sesquiterpenes, which are reported to contain high biological activity (DURAIPANDIYAN et al., 2012; NISHIDA et al., 2005; ZAPATA et al., 2009). Among the low polarity compounds that are most abundant in the *Drimys*

genus, the drimanes sesquiterpenes are the most interesting (MALHEIROS et al., 2005; RODRÍGUEZ et al., 2005, ZAPATA et al., 2009). This class of compounds has a wide spectrum of biological activities and appears to play an important role in plant defense mechanisms (JANSEN; GROOT, 2004). The high herbicidal potential of *D. brasiliensis* observed in the present study may be associated with the sesquiterpenes compounds. However, the presence of sesquiterpenes, particularly in *D. brasiliensis* roots, needs to be confirmed. Thus, this research serves as a starting point for the purification and characterization of the compounds responsible for the phytotoxic effects exerted by the hexane fraction of *D. brasiliensis* root extract. The identification of these phytotoxins may contribute to a better understanding of the allelopathic potential of *D. brasiliensis* and provide interesting opportunities for weed management based on natural products.

## Conclusion

*D. brasiliensis* displayed strong phytotoxicity towards *E. heterophylla* and *P. maximum*. The hexane fraction of the roots showed great potential to inhibit the parameters measured, particularly weeds root growth. The inhibitory effects could also be observed at the cellular level of *E. heterophylla* roots, with a decrease of over 50% in metaxylem cell size. The early growth inhibition effects of the hexane fraction of root extract were similar to the effects produced by the commercial herbicide, thus confirming the potential use of *D. brasiliensis* in weed control.

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