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Allelopathic potential of *Sapindus saponaria* L. leaves in the control of weeds

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ABSTRACT. The objective of this work was to evaluate the allelopathic potential of aqueous extracts of young and mature leaves from *Sapindus saponaria* on diaspore germination and seedling growth of barnyardgrass (*Echinochloa crus-galli*) and morningglory (*Ipomoea grandifolia*). The aqueous extract was prepared in a proportion of 100 g of dried, ground leaves dissolved in 1000 mL of distilled water, resulting in a 10% extract concentrate. Dilutions of this concentrate were made with distilled water to 7.5, 5.0 and 2.5%. In seedling growth tests, we compared the effect of these extracts with the herbicide nicosulfuron. Both extracts of mature and young leaves caused delays and reductions in diaspore germination and seedling length of barnyardgrass and morningglory, with the most intense effects observed at a concentration of 10%. The effects of the young leaf extract were more similar to those observed with the herbicide, demonstrating that leaf maturation stage of *S. saponaria* affects its inhibitory effects on the growth of other plants and that this species is effective in controlling weeds.

Keywords: allelopathy, *Echinochloa crus-galli*, *Ipomoea grandifolia*, soapberry.

Potencial alelopático de folhas de *Sapindus saponaria* L. no controle de plantas daninhas

RESUMO. O objetivo deste trabalho foi avaliar o potencial alelopático do extrato aquoso de folhas jovens e maduras de *Sapindus saponaria* (sabão-de-soldado) na germinação de diásporos e no crescimento de plântulas de capim-arroz (*Echinochloa crus-galli*) e corda-de-viola (*Ipomoea grandifolia*). O extrato aquoso foi preparado na proporção de 100 g de folhas secas e trituradas dissolvidas em 1000 mL de água destilada, produzindo-se o extrato considerado concentrado (10%). A partir deste, foram feitas diluições em água destilada para 7,5; 5,0 e 2,5%. No teste de crescimento de plântulas comparou-se o efeito desses extratos com o herbicida nicosulfuron. Os extratos de folhas maduras e jovens causaram atraso e redução na germinação dos diásporos e no comprimento das plântulas de capim-arroz e corda-de-viola, com efeitos mais intensos na concentração de 10%. No entanto, os resultados obtidos pelo extrato de folhas jovens foram mais similares ao efeito do herbicida, mostrando que o estágio de maturação da folha de *S. saponaria* interfere nos seus efeitos inibitórios e que esta espécie é eficiente no controle das plantas daninhas.

Palavras-chave: alelopatia, *Echinochloa crus-galli, Ipomoea grandifolia,* sabão-de-soldado.

Introduction

As a result of agricultural expansion, Brazil has become the largest consumer of pesticides in the world, accounting for 86% of the Latin American pesticide market (AZEVEDO, 2010). However, the large-scale use of these products has been accompanied by environmental problems, including significant impacts on biota and risks to human health (VYVYAN, 2002). Thus, the search for natural substances that do not possess the disadvantages of synthetic herbicides is of fundamental importance (SOUZA FILHO et al., 2006).

Many techniques are being applied in an attempt to reduce the use of commercial herbicides. These include weed management in crop rotation, systems of sowing between species, use of green manure and other agro-ecological systems (KATO-NOGUCHI, 2003). All of these models are affected by allelopathic relationships between the species involved.

Allelopathic substances are present in all plant tissues, including leaves, flowers, fruit, roots, stems and seeds (GATTI et al., 2004), and may be released by a variety of processes (BONANOMI et al., 2006). However, the distribution of substances is not uniform and; variations depend on the species and plant organ analyzed (WEIR et al., 2004). The main factors that can change the rate of allelochemical production are seasonality, circadian rhythms and maturation stage. Generally, young tissues have a high biosynthetic rate of secondary metabolites. However, there is often an inverse correlation between high metabolic activity and the quantity of allelochemicals produced, especially phenolic derivatives (GOBBO-NETO; LOPES, 2007).

Due to their production of allelochemicals, plants can regulate the microbial community in their immediate vicinity, endure herbivores, encourage symbiotic improvement, change the physical and chemical properties of the surrounding environment and inhibit the growth of plant competition species that can interfere negatively with local culture, causing economic losses to agriculture (PEDROL et al., 2006). These compounds play an important role in determining the diversity, dominance, natural succession of vegetation and plant productivity in agro-ecosystems (YOUNG; BUSH, 2009).

In tropical areas dominated by soils of low fertility and high acidity, there is increasing interest in agro-forestry as an alternative to agricultural expansion. These systems are more balanced from the environmental and sustainability point of view than those based on monocultures. Tree species with allelopathic activity may play a crucial role in the stability of agro-forestry systems, especially with regard to weed control (SOUZA FILHO et al., 2006).

Sapindus saponaria L. (soapberry) is a native arboreous species (Sapindaceae), with regular distribution in the states of the North, Northeast and Midwest. It usually grows in wet places, and is a pioneer species used in landscaping and in models for recovery of degraded areas (ALBIERO et al., 2001). Its phytochemical composition is well-known and includes a great diversity of chemical compounds, such as saponins (PELEGRINI et al., 2008), but few studies have examined the allelopathic potential of this species.

In this context, this study aimed to evaluate the allelopathic potential of aqueous extracts of young and mature leaves of *Sapindus saponaria* on the germination of diaspores and growth of seedlings of *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass) and *Ipomoea grandifolia* (Dammer) O'Donell (morningglory).

Material and methods

Extract preparation

Leaves of *Sapindus saponaria* were collected from 10 trees growing in São Carlos, São Paulo State (22° 02 'S and 47° 52' W) in May (young leaves) and November (mature leaves) of 2008. The morphological criteria used for leaf differentiation included the categorization of young leaves as those presenting a light green color and membranous texture, whereas mature leaves presented a dark green color and papiraceous texture. Collection months were chosen based upon the phenology of the species. The region is characterized by Aw climate (KÖPPEN, 1948), with dry winters (April to September) and wet summers (October to March). Collected leaves were dried at 40°C for 72 hours and ground in an industrial mill.

Aqueous extracts of the leaves were prepared in a proportion of 100 g of dried leaves in 1000 mL of distilled water, producing a 10% (w v^{-1}) extract. The extract was left to sit at 4°C for 30 minutes and then vacuum-filtered (GATTI et al., 2004). The concentrated extract was collected in a beaker, and diluted to 7.5, 5.0 and 2.5% with distilled water.

Analion pHmeter (model PM608) was used to measure pH values and an automatic osmometer (µOsmotte, model 5004) to measure the osmolality. The osmotic potentials of the most concentrated extracts of young and mature leaves were calculated.

Germination bioassays

Bioassays were conducted in Petri dishes with two sheets of filter paper moistened with 5 mL of extract or distilled water (control). The experimental design was completely randomized, using five treatments and four replicates of 30 diaspores of *Echinochloa crus-galli* and *Ipomoea grandifolia*. The seeds of *Ipomoea grandifolia* were scarified in concentrated sulfuric acid for five minutes and then washed in water (VOLL et al., 2010).

The experiment was conducted in a germination chamber at 25°C, under a photoperiod of 12 hours of light followed by 12 hours of dark and a mean irradiance of 12.26 ± 6.49 μmol m^{-2} s^{-1}, similar to the conditions adopted by Concenço et al. (2008) and Mauli et al. (2009). The criterion selected for measuring germination was embryo protrusion, and this was evaluated every 12 hours during the first seven days of the experiment and at intervals of 24 hours thereafter until the stabilization of germination.

Initial, final and mean germination time, germinability, mean germination rate, rate (Maguire's index), coefficient of variation of the germination time, uncertainty and synchronization were calculated according to Ranal and Santana (2006).

To evaluate the influence of the osmotic potential of the extracts, germination bioassays with diaspores of *Echinochloa crus-galli* and *Ipomoea grandifolia* using polyethylene glycol 6000 (PEG

6000) solutions at -0.3, -0.2 and 0 MPa (control) were performed according to recommendations by Gatti et al. (2004). The experiments were completed using the same methodology described for the germination bioassays.

The term diaspore was used throughout the text to designate both types of dispersal units studied. When barnyardgrass was mentioned, caryopsis was used. The term seed was used to describe the dispersal unit of the morningglory.

Seedling growth

For analysis of barnyardgrass and morningglory growth, seedlings with 3 mm-long roots were selected and transferred to transparent plastic boxes (21.0 x 14.3 x 6.0 cm) containing filter paper as substrate that was moistened with 15 mL of water, young or mature leaf extracts or the herbicide nicosulfuron (1.25 L ha^{-1}), under the same conditions adopted for the germination tests. Nicosulfuron is a selective post-emergent herbicide with systemic action and low toxicity, recommended for grasses and some dicotyledon species control (RODRIGUES; ALMEIDA, 2005).

The boxes were kept in a germination chamber at 25°C, with a photoperiod of 12 hours and mean irradiance of 13.38 ± 7.96 μmol m^{-2} s^{-1} (PAR). The experiment design was completely randomized, utilizing four replicates of 20 seedlings. After seven days, the seedlings were classified as normal or abnormal, according to Gatti et al. (2004) and the seedling shoots and primary root lengths were measured in a random sample of 10 seedlings per replicate, using a caliper.

Statistical analysis

Data were statistically analyzed using normality (Shapiro-Wilk) and homogeneity (Levene) tests. When these two assumptions were met, the analysis of variance (ANOVA) was applied, followed by Tukey's test at 0.05 significance. The lack of normality or homogeneity (or both) led to the use of the non-parametric Kruskal-Wallis and Dunn tests to pairwise comparisons at 0.01 significance.

Linear or quadratic regression models were adjusted when the ANOVA *F* for the germination measurements was significant. The goodness of the models was tested at 0.05 significance and evaluated by its coefficient of determination (R^2). For the variables that showed no significant differences between treatments, the means were represented in the figures with their standard deviations. Linear regression equations were submitted to the parallelism (*F*-test) test to verify the null hypothesis that the slopes of the equations were statistically equal, as described by Sokal and Rohlf (1997).

The data from germinability and mean germination rate were submitted to conjoint analysis, since the ratio between the larger and smaller residual mean square was not greater than 7 (PIMENTEL-GOMES, 1990).

Results and discussion

Extracts of *Sapindus saponaria* leaves significantly inhibited the germination of barnyardgrass and morningglory diaspores. Extract from mature leaves increased linearly the initial germination time (1.08 hours for each addition of 0.01 mg mL^{-1} of extract, for barnyardgrass) and mean germination time (1.39 and 1.21 hours for each addition of 0.01 mg mL^{-1} of extract, for barnyardgrass and morningglory, respectively) (Figure 1). As a result, there was a linear decrease in the mean germination rate (0.0003 $hour^{-1}$ for barnyardgrass and 0.0023 $hour^{-1}$ for morningglory, for each 0.01 mg mL^{-1} of extract) and in the rate – Maguire's index (0.0216 diaspore $hour^{-1}$ for barnyardgrass and 0.1063 diaspore $hour^{-1}$ for morningglory, per each additional 0.01 mg mL^{-1} of extract). Comparison of the linear regressions demonstrated that the mean germination time of both species had the same increment trends, whereas for the mean germination rate and rate (Maguire's index) the decrements differed among species, with the largest reductions observed in morningglory (Table 1).

Table 1. *F*-test comparing the linear regression angular coefficients derived from the germination of *Echinochloa crus-galli* and *Ipomoea grandifolia* diaspores subjected to the action of mature and young leaf extracts of *Sapindus saponaria*.

Measurement (unit)	Equation[1]	$F_{calculated}$
Mature leaves		
$\bar{t}$: mean germination time (hour)	y = 58.94 + 1.39x y'= 18.41 + 1.21x	0.8366 [ns]
$\bar{v}$: mean germination rate ($hour^{-1}$)	y = 0.0169 – 0.0003x y'= 0.0549 – 0.0023x	52.76*
RATE: Maguire's index (diaspore $hour^{-1}$)	y = 0.4913 – 0.0216x y'= 1.79 – 0.1063x	77.64*
Young leaves		
G: germinability (%)	y = 88.67 – 7.53x y' = 96.67 – 7.94x	0.1534 [ns]
CV_t: coefficient of variation of the germination time (%)	y = 33.53 – 2.04x y' = 89.02 – 5.77x	11.98*
RATE: Maguire's index (diaspore $hour^{-1}$)	y = 0.4254 – 0.0382x y' = 1.57 – 0.1739x	99.01*

[1]H_0: $ß_1 - ß_2 = 0$, where $ß_1$ and $ß_2$ are the parameters of the angular coefficients of the equations. y: *Echinochloa crus-galli*; y': *Ipomoea grandifolia*. [ns]: angular coefficients for the two species do not differ significantly. *angular coefficients for the two species differ significantly ($F_{calculated} > F_{tabulated}$).

The initial time of germination for morningglory seeds increased only at an extract concentration of 7.5%. The germinability of barnyardgrass caryopses decreased linearly 2.26% for each addition of

0.01 mg mL^{-1} of extract, while the coefficient of variation of the germination time displayed quadratic regression, with the minimum estimated value of 25.46% at an extract concentration of 10% (Figure 1), indicating that the few caryopses germinated in this concentration had similar physiological characteristics.

The young leaf extracts of *Sapindus saponaria* strongly inhibited germination, especially in morningglory seeds. There was a linear decrease in the germinability (7.53% for barnyardgrass and 7.94% for morningglory for each 0.01 mg mL^{-1} of extract) and in the coefficient of variation of the germination time (2.04% for barnyardgrass and 5.77% for morningglory for each 0.01 mg mL^{-1} of extract), indicating lower dispersion around the mean germination time at an extract concentration of 10% (Figure 2). This homogeneity of diaspores can be confirmed by the low uncertainty value at a 10% concentration, noting that allelochemicals can select diaspores with high vigor. There was also a linear decrease in the mean germination rate (0.0039 hour^{-1} for each 0.01 mg mL^{-1} of extract, for morningglory), rate (0.0382 diaspore hour^{-1} for barnyardgrass and 0.1739 diaspore hour^{-1} for morningglory for each 0.01 mg mL^{-1} of extract), uncertainty (0.054 bit for each 0.01 mg mL^{-1} of extract for barnyardgrass) and synchrony (0.036 for each 0.01 mg mL^{-1} of extract for morningglory) (Figure 2). For barnyardgrass caryopses, the synchrony values showed quadratic regression, with a maximum estimated value of 0.2295 at an extract concentration of 3.6% (Figure 2). The uncertainty of the germination process of morningglory seeds had a higher value (1.92 bits) at a concentration of 5.5% (Figure 2).

For both target species, the synchrony of the germination process had low and near zero values at a *Sapindus saponaria* young leaf extract concentration of 10%, indicating a lack of overlapping germination at the same time. The two weed species had similar decrements in germinability, but for the coefficient of variation of the germination time and mean germination rate, the largest decrease occurred for morningglory seeds (Table 1).

For the germination process, regressions could not be applied to variables that did not present significant differences among treatments of young and mature leaf extracts, so only the means and standard deviation of these variables are shown in Figures 1 and 2.

Similar results of inhibition of the germination process for barnyardgrass caryopses were recorded with the use of aqueous extracts of *Secale cereale* L. leaves (BURGOS; TALBERT, 2000), *Dicranopteris linearis* (Burm. f.) Underw. (CHONG; ISMAIL, 2006) and *Amaranthus hypochondriacus* L. (TEJEDA-SARTORIUS; RODRIGUES-GONZALES, 2008). For morningglory seeds, extracts from *Leucaena leucocephala* (Lam.) de Wit leaves did not change germination, but did affect seedling growth (MAULI et al., 2009).

Conjoint analysis showed that the young leaf extract of *Sapindus saponaria* exhibited a strong inhibitory effect on the germinability and mean germination rate of diaspores of both weed species. In general, morningglory seeds had higher germinability and mean germination rate than barnyardgrass caryopses, but this result may be explained by the scarification to which the morningglory seeds were subjected (Figure 3).

Allelopathic effects of the extract on morningglory seedlings were strongly inhibitory (Figure 4). Furthermore, the weed seedlings were more sensitive to the effects of young leaf extracts than to those of mature leaf extracts, except in the case of shoot length of morningglory seedlings (Figure 4). The root was the organ that responded most dramatically to allelochemical treatment and necrosis was the most commonly observed symptom.

The inhibitory effects of allelochemicals on root development are similar to the damage caused by natural detergents, such as saponins. These substances are present in *Sapindus saponaria* extracts, as reported by Pelegrini et al. (2008), and they act to reduce the respiratory activity due to lower diffusion of oxygen through the seed coat, inhibiting germination and plant growth (MARASCHIN-SILVA; AQÜILA, 2005).

Reduction in root growth is one of the first apparent effects of allelochemical exposure and it is associated with premature lignification of cell walls (SUZUKI et al., 2008). The effects of *Sapindus saponaria* young leaf extracts were similar to those induced by herbicide exposure, demonstrating the efficiency of this tree species in controlling the weeds examined in this study.

Nicosulfuron is a member of the sulfonylurea family of herbicides. Sulfonylurea herbicides control weeds by inhibiting the plant enzyme acetolactate synthase (ALS), which catalyzes the first step in the synthesis of the branched-chain amino acids (valine, leucine and isoleucine). Inhibition of this enzyme leads to leaf chlorosis, necrosis and reduced growth (RODRIGUES; ALMEIDA, 2005). Sulfonylurea herbicides are somewhat selective, differentially affecting plants according to their rates of metabolism, absorption and translocation of the product (SILVA et al., 2005).

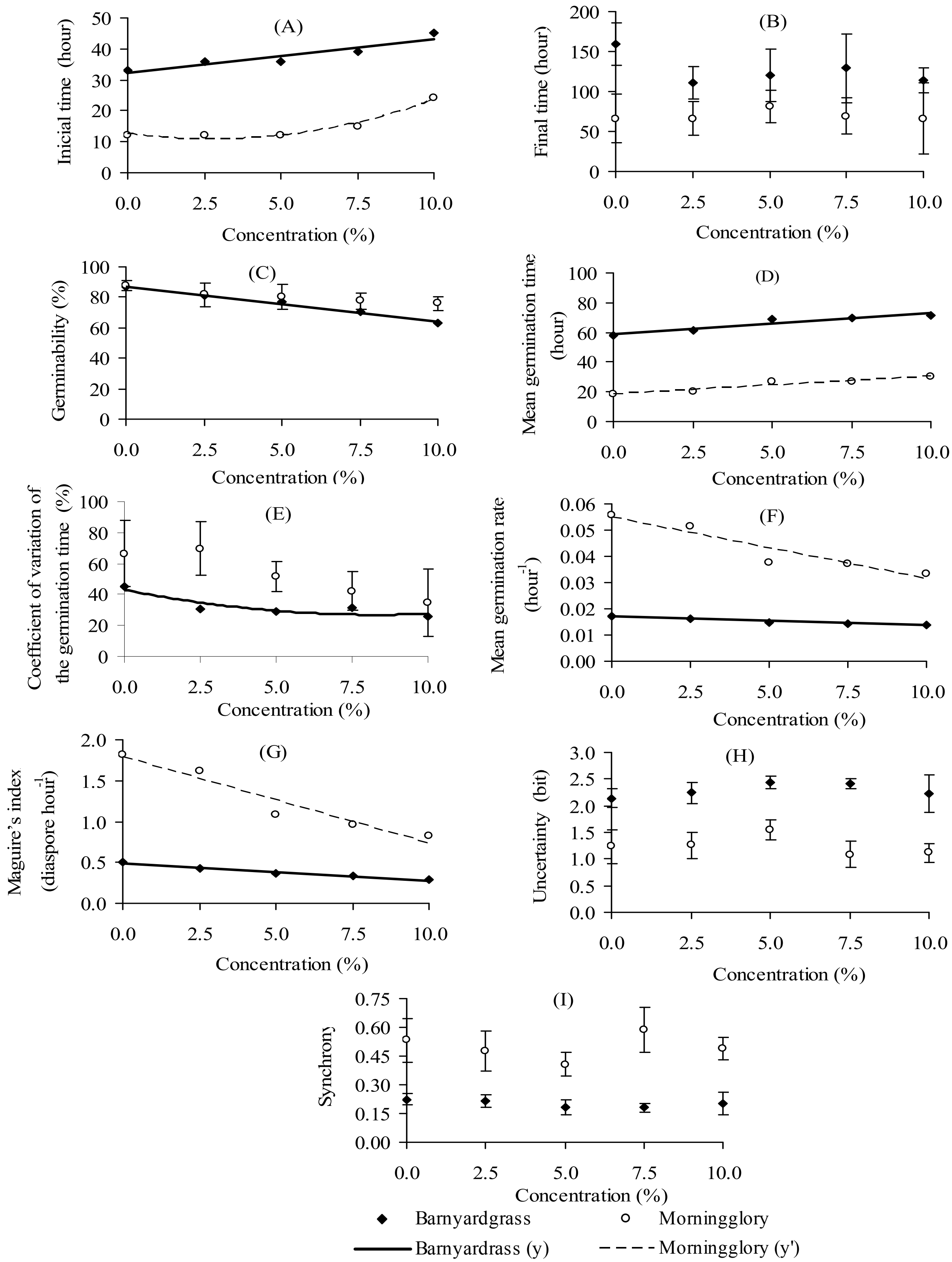

Figure 1. Germination of *Echinochloa crus-galli* (barnyardgrass) and *Ipomoea grandifolia* (morningglory) diaspores in different mature leaf extract concentrations of *Sapindus saponaria*. Equations obtained by regression analysis: (A) $y = 32.40 + 1.08x$, $R^2 = 88.04\%$; $y' = 12.60 - 1.32x + 0.24x^2$, $R^2 = 96.67\%$; (C) $y = 87.00 - 2.26x$, $R^2 = 99.06\%$; (D) $y = 58.94 + 1.39x$, $R^2 = 90.65\%$; $y' = 18.41 + 1.21x$, $R^2 = 91.75\%$; (E) $y = 43.01 - 3.86x + 0.2310x^2$, $R^2 = 80.35\%$; (F) $y = 0.0169 - 0.0003x$, $R^2 = 89.77\%$; $y' = 0.0549 - 0.0023x$, $R^2 = 88.99\%$ (G) $y = 0.4913 - 0.0216x$, $R^2 = 97.63\%$; $y' = 1.79 - 0.1063x$, $R^2 = 93.23\%$. Vertical bars represent the standard deviation of the mean.

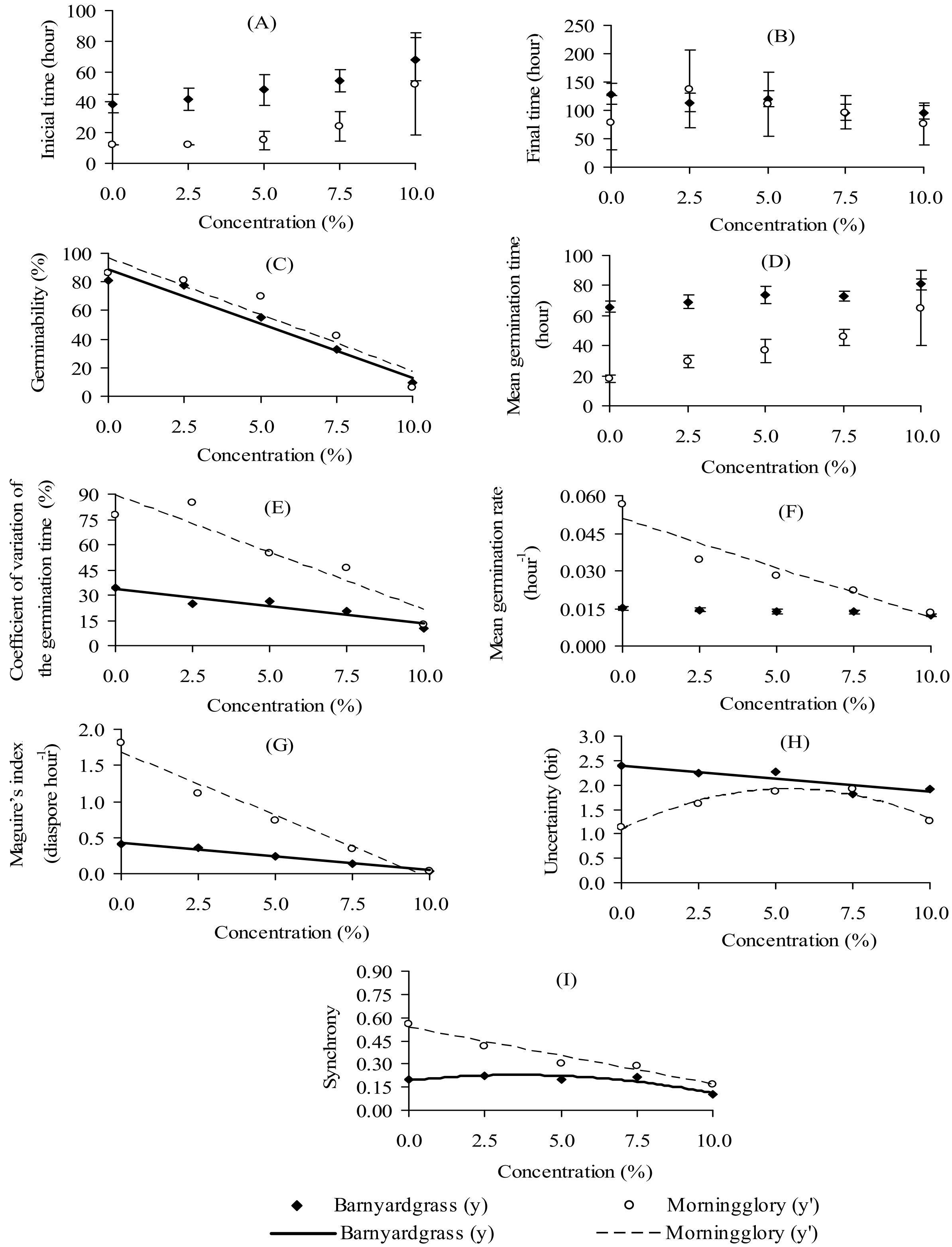

Figure 2. Germination of *Echinochloa crus-galli* (barnyardgrass) and *Ipomoea grandifolia* (morningglory) diaspores in different young leaf extract concentrations of *Sapindus saponaria*. Equations obtained by regression analysis: (C) $y = 86.67 - 7.53x$, $R^2 = 95.85\%$; $y' = 96.67 - 7.94x$, $R^2 = 89.47\%$; (E) $y = 33.53 - 2.04x$, $R^2 = 87.63\%$; $y' = 89.02 - 5.77x$, $R^2 = 87.05\%$; (F) $y' = 0.0507 - 0.0039x$, $R^2 = 91.74\%$; (G) $y = 0.4254 - 0.0382x$, $R^2 = 98.66\%$; $y' = 1.57 - 0.1739x$, $R^2 = 97.25\%$; (H) $y = 2.39 - 0.0540x$, $R^2 = 77.20\%$; $y' = 1.08 + 0.308x - 0.028x^2$, $R^2 = 94.10\%$ (I) $y = 0.1927 + 0.0203x - 0.0028x^2$, $R^2 = 83.77\%$; $y' = 0.530 - 0.036x$, $R^2 = 95.10\%$. Vertical bars represent the standard deviation of the mean.

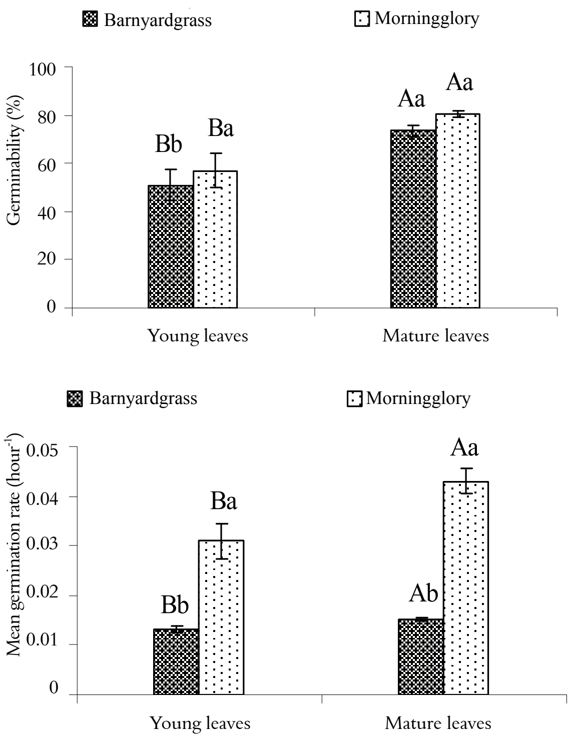

Figure 3. Germinability and mean germination rate of *Echinochloa crus-galli* (barnyardgrass) and *Ipomoea grandifolia* (morningglory) diaspores in young and mature leaf extracts of *Sapindus saponaria*. Means followed by the same capital letters for organs and small letters for species do not differ significantly by Tukey's test at 0.05% probability. Vertical bars represent the standard error of the mean.

According to Nascimento et al. (2007), extracts of young leaves of *Pouteria torta* (Mart.) Radlk. were more potent than extracts of mature leaves in inhibiting the germination and seedling growth of lettuce. Another study found that the density of trichomes containing flavonoids in young leaves and/or near to the reproductive organs of *Paulownia tomentosa* (Thunb.) Steud. was higher than in mature leaves (KOBAYASHI et al., 2008).

Young leaves are rich in nutrients, providing a large pool of assimilates and plant hormones. These features, while beneficial to plant health, may also attract herbivores. Herbivore preference for young leaves may also be related to the leaves' higher rate of cellular division and growth and the lack of a secondary lignified cell wall, which makes them softer, facilitating their mastication and digestion (VARANDA et al., 2008). Young leaves possess a variety of characteristics that reduce herbivory damage. Some are more pubescent, containing larger concentrations of tannins and other secondary metabolites than mature leaves (VARANDA et al., 2008).

The crude extracts of the young and mature leaves of *Sapindus saponaria* had pH values of 6.55 and 6.21, respectively. Considering that germination and seedling development are negatively affected by conditions of extreme acidity or alkalinity (GATTI et al., 2004), the pH of young and mature leaf extracts of *Sapindus saponaria* probably does not interfere with these processes.

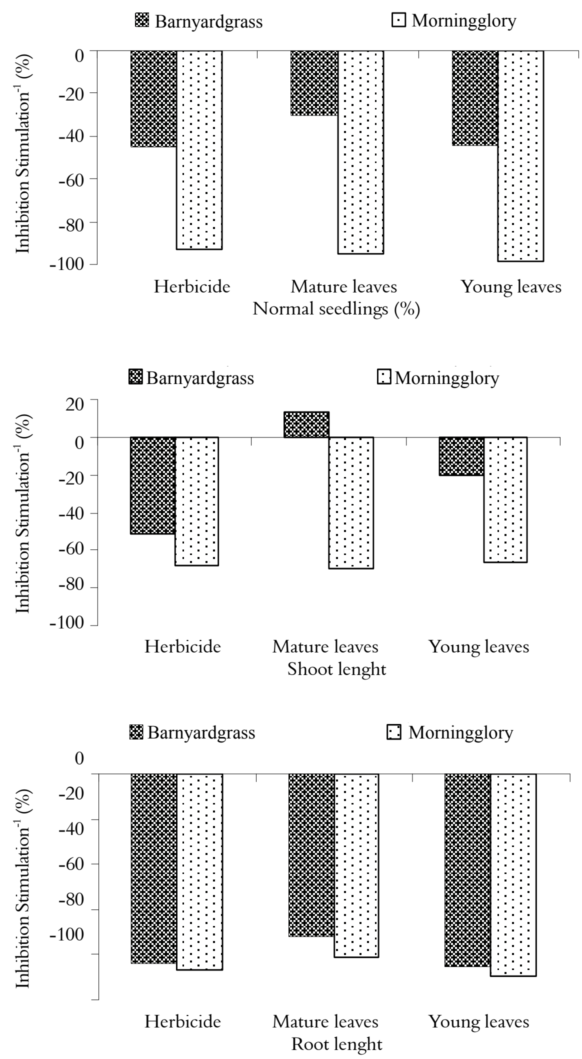

Figure 4. Effects of herbicide, young and mature leaf extracts of *Sapindus Saponaria* on the percentage of normal seedlings, shoot and root length of *Echinochloa crus-galli* (barnyardgrass) and *Ipomoea grandifolia* (morningglory) seedlings.

The osmotic potentials of the young and mature leaf extracts of *Sapindus sapon*aria were -0.25 and -0.32 MPa, respectively. In PEG-6000 solutions, 80% of barnyardgrass caryopses germinated at osmotic potentials of 0 and -0.2 MPa, and 63% of caryopses germinated in a solution of -0.3 MPa. This result suggests a possible interference of the young leaf extract osmotic potential in the germination of barnyardgrass caryopses. However, 82, 80 and 79% of morningglory seeds germinated at potentials of 0, -0.2 and -0.3 MPa, respectively. Considering that solutions with osmotic potentials of -0.2 for barnyardgrass caryopses and -0.3 MPa for morningglory seeds did not interfere significantly with germination, we can infer that the reduction in the germinability of diaspores occurred specifically as a result of the presence of substances with allelopathic activity in these extracts (Figures 1 and 2).

Conclusion

The allelopathic potential of *Sapindus saponaria* leaf extracts varies according to the target species and displays concentration-dependent inhibitory effects. The maturation stage of *Sapindus saponaria* leaves interferes with these allelopathic effects, since the young leaf extract demonstrated stronger inhibition than the mature leaf extract of the germination process and seedling development of barnyardgrass and morningglory.

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