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Time of exposure and oil dosage affecting biomass production of the Amazonian semi-aquatic grass *Echinochloa polystachya*

O tempo de exposição e a dosagem de óleo afetando a produção de biomassa do capim semi-aquático amazônico Echinochloa polystachya

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Abstract

The effects of Urucu crude oil on growth and biomass production of *Echinochloa polystachya* were studied experimentally. Young plants contaminated with 5 oil doses (0 to 2.64 $l.m^{-2}$) were monitored at 1, 5, 10, 15 and 20 days after exposure. A significant reduction was observed in leaf humidity and the ratio of live aerial/total biomass related to the increase in oil dosage. Increased exposition time caused a reduction in the number of leaves, aerial biomass, proportion of live aerial/total biomass, total biomass, and an increase of dead aerial biomass. However, root biomass and leaf length were not affected by exposure to oil. The low tolerance of *E. polystachya* to Urucu crude oil has lead to the mortality of 90% of the plant population in 20 days at the 2.64 l treatment. The death of young individuals of *E. polystachya* by relatively low dosages of Urucu oil indicates that, in the case of an oil spill in the Amazon varzea, this species would be severely affected, endangering, consequently, the entire environment.

Key words: floodplains, aquatic macrophytes.

Resumo

Os efeitos do petróleo cru de Urucu sobre o crescimento e a produção de biomassa da herbácea aquática amazônica *Echinochloa polystachya* foram estudados experimentalmente em casa de vegetação. Plantas jovens de *E. polystachya* foram contaminadas com 5 dosagens de petróleo (0 to 2,64 $L.m^{-2}$) e avaliadas após 1, 5, 10, 15 e 20 dias da exposição. Ao término do experimento foi verificada uma significativa diminuição da proporção de biomassa aérea viva/total, da umidade das folhas e aumento do número de folhas com comprometimento fitossanitário, em resposta ao aumento da dosagem do petróleo. O aumento do tempo de exposição levou à diminuição do número de folhas, biomassa aérea, proporção de biomassa aérea viva/total, biomassa total e um aumento do número de folhas comprometidas e biomassa aérea morta. Contudo, a biomassa de raízes e o comprimento das folhas não foram afetados pela exposição ao petróleo. A baixa tolerância de *E. polystachya* ao petróleo de Urucu levou à mortalidade de 90% da população de mudas em 20 dias com o tratamento de 2,64 $L.m^{-2}$. A perda de indivíduos jovens de *E. polystachya* em doses relativamente baixas do petróleo de Urucu indica que, no caso de um derramamento de óleo na várzea amazônica, esta espécie seria severamente afetada e, consequentemente, todo o ambiente.

Palavras-chave: áreas alagáveis, macrófitas aquáticas.

Introduction

Among the many factors endangering wetlands around the world, oil spills are amongst the most serious (Val & Almeida-Val 1999). Petroleum may change species composition, diversity and structure of communities, owing to its specific effect on the dominant species in a given area (Burk 1977; Lopes 2007), since the hydrocarbons act at the chemical and physical levels on plants (Pezeshki *et al*. 2000). Urucu is the region in the Brazilian Amazon where intense petroleum extraction has taken place over the last decade. The petroleum produced in this area is classified as light (ANP, 2008), a type of oil that acts at the cellular level, changing membrane permeability or interrupting various plant metabolism processes (Pezeshki *et al*. 2000). While heavy oils cause physical damage resulting in asphyxia and interruption of gas exchange, the effects of light oils are immediately toxic to plants.

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The study of the effect of crude oil on aquatic macrophytes is essential to predict the effect of oil spills on biodiversity, contributing as well to establish the techniques and costs for the recovery of affected areas. Wild aquatic grasses are often used for the recovery of contaminated areas due to their intense root ramification providing a wide surface for the microbiota, facilitating plant-microbiota interplay (Hutchinson *et al*. 2001; Glick 2003). The semi-aquatic grasses growing in the varzea floodplains near Manaus are of major importance since they form huge stands dominated by the Poaceae species *Echinochloa polystachya* (H.B.K.) Hitchcock, *Hymenachne amplexicaulis* (Rudge) Nees, *Leersia hexandra* Sw., *Oryza perennis* Moench and *Paspalum repens* P.J. Bergius (Junk & Piedade 1997). *Echinochloa polystachya* grows in large monospecific stands on the floodplains of the Amazon River, having its life cycle controlled by the flood pulse (Junk *et al.* 1989). Combining sexual reproduction and vegetative propagation by regrowth during low water levels, the species constitutes the most productive plant population known, reaching about 100 $t.ha^{-1}$ (dry matter per year) (Piedade *et al.* 1991, 1992).

Studies with *E. polystachya* in Mexico in soils contaminated with weathered petroleum hydrocarbons have shown that this plant is able to survive in these environments, although it may present a reduction of up to 53% in biomass (Rivera-Cruz *et al.* 2002; Rivera-Cruz & Trujillo-Narcía 2004). Previous studies with *E. polystachya* exposed to Urucu oil to test the effect of low dosages on regrowth of the species have shown a decrease of 50% in regrowth capacity at 0.46 $l.m^{-2}$ (Lopes & Piedade 2009). In addition, the period of the year when the petroleum was added changed the response of plants to the dosage applied, due to differences in environmental temperatures, with more severe negative effects during the higher temperature period (Lopes & Piedade 2010). Finally, the effect of Urucu oil on young *E. polystachya* had major negative effects, with 50% of population mortality attained in only four days, at 0.47 $l.m^{-2}$ (Lopes *et al.* 2009). However, the effect of oil on biomass production over time is still to be elucidated.

In this study the effect of Urucu oil on young individuals of *E. polystachya* was investigated to evaluate the influence of time of exposure and oil dosage on biomass production of the species. We hypothesized that increased exposure time and dosage of oil causes a reduction in growth and biomass production of *E. polystachya*, which may explain previous findings that show the susceptibility of the species in terms of regrowth and survival under addition of oil.

Material and Methods

Echinochloa polystachya was manually collected, in May 2006, on the island Ilha de Marchantaria (03°15'S, 60°00'W), in the Central Amazon, Amazonas-Brazil. The soil for the experiment was sampled in the non-flooded areas of the same location. The experiment was performed in the greenhouse of the INPA/Max-Planck Project; the water used for irrigation was taken from an artesian well.

Crude oil from the Urucu Petroliferous Base (Amazonas, Brazil) was used in the experiment. This oil is considered light, with 45.8 API degrees (ANP 2008). See Table 1 for oil composition (Petrobras 1997).

Parts of the culm of mature plants of *E. polystachya* were collected and from each one a 15 cm portion containing one node was taken. The propagules were planted in vases with 2 liters of varzea soil (70.5% clay, 17.92% silt, 8.97% coarse sand, 2.61% fine sand). After 30 days, oil was placed on the soil surface in each recipient, without direct contact with leaves. The dosages of petroleum (0, 0.08, 0.32, 1.32 and 2.64 $l.m^{-2}$) were randomly distributed in herbaceous plants.

Sample units consisted of 250 plastic vases (20 cm in diameter by 18.5 cm in height) distributed in factorial experiments in a completely randomized design 5×5 (oil dosage, time of exposure) with 10 replicates per treatment.

Table 1 – Composition of Urucu crude oil used in the experiment (Petrobras 1997).

Motor octane number (distillation cut point 16–180°C)	38.0
Hydrocarbon types (% of total; distillation cut point 16–180°C)	
Paraffins	64.8
Napathelenes	20.2
Aromatic	15.0
Hydrocarbon types (% of total; distillation cut point 144–244°C)	
Saturates	85.7
Aromatics	13.2
Oleffins	1.1

Toxicity tests were performed according OECD 2006 Protocol. The following parameters of plant response to toxicity were analyzed: mortality (the plant was considered dead when less than 30% of total surface was green). Mortality was determined by the number of dead plants in each group of 10 individuals, according to each treatment. The mortality rate in each treatment was used to indicate species sensitivity to crude oil. The number of healthy leaves and their phytosanitary condition (leaves with less than 30% of green surface) indicate plant investment in maintaining leaf production. Furthermore, leaf length was verified, indicating plant growth during the experimental period. Aerial biomass was harvested after 1, 5, 10, 15 and 20 days, divided into dead and live components, and dried at 65ºC to constant weight. Root biomass was determinate collecting the roots that were washed and dried in a oven at 65ºC, to constant weight, without separating live and dead material. Total biomass was calculated using the following equation: TB = lab + dab + rb; where: TB = total biomass, lab = live aerial biomass, dab = dead aerial biomass, and rb = root biomass. The ratio of aerial live/total biomass was calculated as an indicator of oil impact on the plants. Water percentage in leaves (H%) was measured in order to estimate the value of H%, using the equation: H%=(WW-DW)/ WW)*100; where: WW = wet weight (g), DW = dry weight (g), and H% is the percentage of humidity in each fraction of plant. Humidity was used as an indicator of water stress in the plant.

Statistical analysis was performed using Systat 10.2 software (Systat Inc 2002). Plant survival was evaluated using the nonparametric estimator Kaplan-Meier. This method estimates the probability of occurrence of events over a short term period, days or months, with intervals not necessarily equally spaced (Ayres & Ayres Jr. 1998).

The effect of the treatments was stated using a general linear model. To analyze the effect of the dosage and time of exposition an Analysis of Variance (ANOVA) was used. For this analysis the dead plants and the ones exposed to the 2.64 l dosage,in treatments of 10 and of 20 days, were exlcluded, due to high mortality. Tukey's test was performed when there was a significant difference among the treatments (α<0.05). Biomass data were normalized using log (g) with the exception of the aerial biomass data, to follow the assumptions of the methods applied.

Results and Discussion

The results show that when applied to the soil the Urucu crude oil promotes severe impacts on *E. polystachya* in controlled experimental conditions, with high rates of mortality at all oil dosages (Tab. 2). These results corroborate the findings of Lopes & Piedade (2009) and Lopes & Piedade (2010), in experiments of regrowth of *E. polystachya* with petroleum addition. For *E. polystachya* Lopes *et al.* (2009) calculated the LD50 (median lethal dose) in four days of exposure, at 0.47 $l.m^{-2}$ of Urucu oil; in the present study 20% of mortally occurred in 5 days of exposure at the treatment of 0.32 l, confirming the high toxicity of this oil.

The growth of aquatic macrophytes is directly proportional to the intensity and amount of light and available nutrients (Petrucio & Esteves 2000). In the present study *E. polystachya* was planted in vases with varzea soil rich in nutrients (Furch & Junk 1997) as substrate. The vases were placed in a greenhouse with transparent roof to hinder the rain, but allowing natural solar radiation. The plants were watered daily to prevent water stress. Therefore, since the offered conditions were ideal for development, differences in results between treatments with varying oil dosages may be the result of its effect on the plants.

Soon after the application of Urucu crude oil in the soil a darkening at the base of the leaves was observed, in plants at dosages of 1.32 and 2.64 l, leading to the wilting and subsequent death of the plants. Mortality observed in *E. polysthachya* in this experiment was higher than mortality reported in other experiments using higher dosage of different oils and other species of plants (Pezeshki, & DeLaune 1993; Lin & Mendelssohn 1996; Lin *et al.* 2002), or

Table 2 – Rate of mortality (%) of *E. polystachya* after crude oil exposure according to applied oil dosages and time of exposure (days).

	Petroleum dosage ($l.m^{-2}$)				
Time	0	0.08	0.32	1.32	2.64
1	0	0	0	0	20
5	0	10	20	20	70
10	0	20	40	20	100
15	0	30	50	80	80
20	0	30	40	50	90

using Urucu oil applied to *Eichhornia crassipes* (*Mart.*) Solms (Pontederiaceae), that survived for 35 days in 15.89 $l.m^{-2}$ (Lopes *et al.* 2009).

Survival analysis showed that the dosage of oil caused a significant reduction in survival of *E. polystachya* ($t_{(245)} = 4.141$, $p < 0.001$) throughout the time of exposure. Factorial analysis showed that the death of *E. polystachya* increased by 60% when exposure time increased from 5 to 20 days and 20% when dosage increased from 0.08 l to 2.64 l of oil. The interaction of these two factors resulted in an increase of 20% in plant mortality.

The sensitivity of the plants to different types of oil is reported by many authors (Mendelssohn & Mckee 1988; Pezeshki *et al.* 2000; Lopes & Piedade 2009, 2010). As some plants are very resistant to pollutants, differences of sensitivity may lead to a shift in the floristic composition of affected areas. The present study showed that Urucu crude oil when spilled on the surface of soil causes the death of 90% of the plants of *E. polystachya* in 20 days of exposure at 2.64 $l.m^{-2}$, showing the high sensitivity of this species to this oil. In a similar study Lin & Mendelssohn (1996) had observed that *Spartina patens* (Aiton) Muhl. (Poaceae) is more sensitive than *S. alterniflora* Loisel when exposed to crude oil of Louisiana, resulting in the death of all plants in dosages over 8 $l.m^{-2}$, a dosage higher than the one used in this experiment.

Increase in leaf number was affected by oil dosage and time of exposure (Tab. 3). Analysis of each time separately has shown that after 10 days of exposure there was a significant reduction in leaf number at treatment of 1.32 $l.m^{-2}$ ($F_{(3, 28)} = 4.270$, $p = 0.013$), and after 15 days of exposure the reduction in leaf number was higher ($F_{(4, 21)} = 2.932$, $p = 0.045$; Fig. 1a). There was a significant increase of leaves with phytosanitary problems in relation to dosage and time of exposure (Tab. 3).

The analysis of dosage effect of Urucu crude oil in the different times showed that, compared to the control, the number of leaves with phytosanitary problems increased after 1 day of exposure ($F_{(4, 43)} = 2.986$, $p = 0.029$) in the 2.64 $l.m^{-2}$ treatment. After 5 days of exposure there was a significant difference in the number of leaves with phytosanitary problems in the dosage of 1.32 $l.m^{-2}$ ($F_{(4, 33)} = 3.050$, $p = 0.03$; Fig. 1b). On the other hand, leaf length was not affected by oil dosage or by exposure time (Tab. 3), although the dosage presented a significant effect after 5 days of exposure ($F_{(4, 33)} = 3.104$, p = 0,028; Fig. 1c).

Table 3 – ANOVA results of crude oil dosage effect, of time of exposure and of interaction among these factors on biometric parameters of the plants. d.f. (degrees of freedom).

Dependent Variable	Factor	d.f.	*F*	*p-valor*
Nº of leaves	Dose	3	9.655	<0.0001
	Time	4	4.305	0.003
	Interaction	12	0.718	0.732
	Error	139		
Nº of dead leaves	Dose	3	8.354	<0.0001
	Time	4	39.226	<0.0001
	Interaction	12	1.197	0.291
	Error	139		
Leaf length	Dose	3	0.30610	0.82095
	Time	4	1.80486	0.13129
	Interaction	12	0.68561	0.76314
	Error	139		

Figure 2a shows that aerial biomass did not show a significant difference in relation to the dosage, although a significant increase with exposure time was observed (Tab. 4); the difference between treatments and control were significant after 5 days of exposure at 1.32 $l.m^{-2}$ of oil ($F_{(4, 33)} = 3.434$, p = 0.019) and after 15 days at 2.64 l treatment ($F_{(4, 21)} = 18.470$, p < 0.0001). Additionally, there was a significant reduction in the humidity of leaves (H%) related to oil dosage and exposure time (Tab. 4); 86% of humidity was found in the treatment of 1.32 $l.m^{-2}$ after 1 day of exposure, and 80% after 20 days of exposure. The reduction of leaf humidity as a consequence of oil exposure suggests water stress, which may contribute to plant mortality.

Root biomass did not show a significant difference in response to oil dosage, or time of exposure (Tab. 4). Lin *et al.* (2002) found a decrease of root biomass of *S. alterniflora* by Nº 2 oil in dosage of 0.3 $l.m^{-2}$ of soil. However, the relation between root biomass and oil dosage was not found in the present study which may have occurred due to exposure time of only 20 days, and because *E. polystachya* has a low mass of roots (Piedade *et al.* 1991). On the other hand, in the long term, a much stronger effect of soil contamination on the root system was found by Lopes *et al.* (2009) for the same species. This happens because soil texture affects residual dosage of oil in soil and in vegetation due to size difference and pore distance. Sandy substrate with larger pores allows a faster and deeper penetration of pollutants than that of fine texture soil (Pezeshki *et al.* 2000). In the Amazon, oil probably would be accumulated on varzea soil which is mostly composed of silt (granule

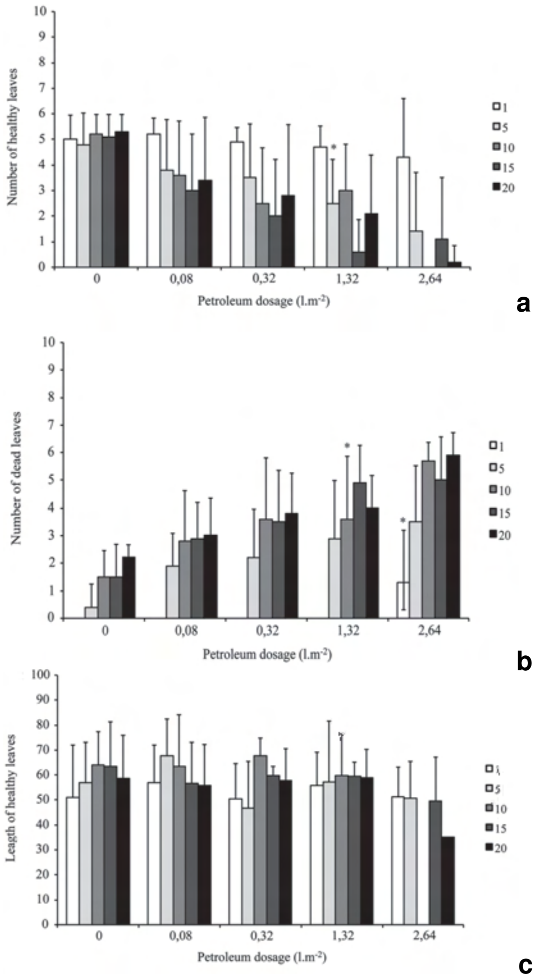

Figure 1 – Effect of Urucu's oil over Echinochloa polystachya at: a) the number of healthy leaves (means ± SD); b) the number of dead leaves (means ± SD); c) the length of healthy leaves (means ± SD). * significant difference (± = 0.05) compared to the control.

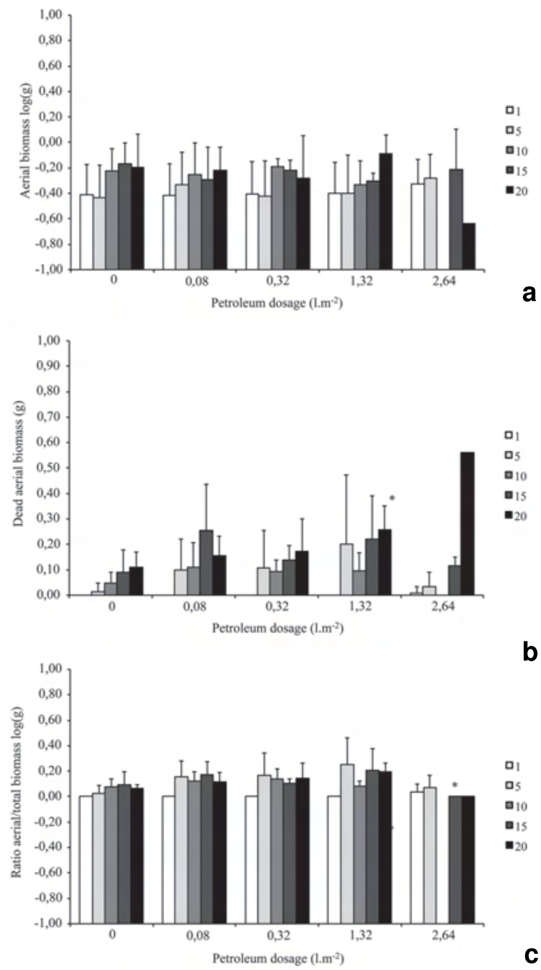

Figure 2 – Effect of Urucu crude oil on *Echinochloa polystachya* at: a) the aerial biomass (means ± SD); dead aerial biomass (means ± SD); c) the ratio of aerial live/total biomass (means ± SD).* significant difference ($\alpha = 0.05$) compared to the control.

of 2 a 63µm) and clay (granule < 2 µm), sediments rich in essential elements for the plants (Irion *et al.* 1997). Such accumulation of oil would result in extended effects on vegetation.

The production of dead aerial biomass of *E. polystachya* was affected by oil dosage and time of exposure (Tab. 4). The oil caused an increase in production of dead aerial biomass after 15 days ($F_{(4, 42)} = 2.932$, $p = 0.045$), with a significant increase in the treatment of 1.32 l after 20 days ($F_{(3, 24)} = 3.264$, $p = 0.039$, Fig. 2b).

There was no significant effect of oil dosage on the total biomass of *E. polystachya*, although there was a significant difference between exposure times (Tab. 4). However, when the effect of Urucu crude oil on the rate of aerial live/total biomass was analyzed the effect of oil dosage, time of exposure and the interaction among such factors resulted in an increase of the proportion of aerial live biomass in relation to the total at the control and the reduction in the remaining treatments with the increase of time (Tab. 4). Moreover there was a significant effect of this reduction after 15 days of exposure in the treatment at 2.64 $l.m^{-2}$ ($F_{(4, 21)} = 9.747$, $p < 0,0001$; Fig. 2c).

Besides the occurrence of high mortality, surviving plants had their growth compromised.

Table 4 – Results of ANOVA two-away for the dependent variables in relation to the factors, oil dosage (Dose), time of exposure (Time), and the interactions between the two factors (interaction). d.f. (degrees of freedom).

Dependent Variable	Fator	d.f.	*F*	*p*
Aerial biomass	Dose	3	0.058	0.982
	Time	4	5.368	0.000
	Interaction	12	0.492	0.917
	Error	139		
Water percentage in leaves (H%)	Dose	3	33.968	0.015
	Time	4	136.619	0.000
	Interaction	12	9.596	0.437
	Error	139	9.436	
Roots biomass	Dose	3	0.316	0.813
	Time	4	12.055	0.000
	Interaction	12	0.247	0.995
	Error	139		
Dead aerial biomass	Dose	3	6.767	<0.0001
	Time	4	15.893	<0.0001
	Interaction	12	1.353	0.196
	Error	139		
Total biomass	Dose	3	2.375	0.073
	Time	4	4.137	0.003
	Interaction	12	0.432	0.948
	Error	139		
Aerial/total biomass	Dose	3	5.031	0.002
	Time	4	1.178	0.323
	Interaction	12	0.758	0.693
	Error	139		

This was highlighted by the decrease in the ratio of aerial live/total biomass, which was 57.2% higher in control than in treatment with 1.32 $l.m^{-2}$ of oil after 15 days of exposure. Lin & Mendelssohn (1996) demonstrated that a dosage of 8 $l.m^{-2}$ of crude oil of Louisiana was needed for a decrease in the proportion of live/dead biomass in *S. alterniflora*, after one year of application. In *E. polystachya* at the present study, a dosage of 2.64 $l.m^{-2}$ was enough to cause a significant decrease in the proportion of live aerial/total biomass after just 15 days of exposure.

The direct effects of plant contamination by oil relate mainly to the decomposition of contaminated biomass which causes alterations in the soil microbiota, affecting remineralization of nutrients (Pezeshki *et al.* 2000). Lin & Mendelssohn (1996) verified that oil from Louisiana, in dosages of 16 and 24 $l.m^{-2}$ soil, on *S. alterniflora* and *S. patens* lead to the increase of dead aerial biomass after one year of application. In the present study, dosages of 2.64 $l.m^{-2}$ in 20 days of exposure were sufficient to increase the dead aerial biomass of *E. polystachya*, confirming once more the high sensitivity of this species to the Urucu oil.

The removal of young individuals of *E. polystachya* by relatively low dosages of Urucu oil indicate that, in the case of an oil spill in the Amazon varzea during the period of regrowth, this species would be severely affected. Consequently, nutrient cycling in floodplain areas would be drastically altered, since this herbaceous plant is the main species responsible for biomass production in those environments (Piedade *et al.* 1992). Owing to *E. polystachya*'s role in the carbon cycle of the varzea ecosystem (Piedade *et al.* 1991), a severe oil spill of Urucu oil, will have fatal implications on the carbon balance of these areas and, eventually, in other regions of the Amazonian floodplains.

Dosage and time of exposure are very important in the determination of oil impact on vegetation. Low dosages of oil may cause the reduction of leaf number and live aerial/total biomass of *E. polystachya*, whilst high dosage may even cause plant death. Low dosage of Urucu oil, such as 0.08 and 0.032 $l.m^{-2}$, although causing the death of some plants did not have other significant effects on them. Moderate dosages, such as 1.32 $l.m^{-2}$ caused reduction in the number of leaves and increase of number of leaves with phytosanitary problems. Higher dosages, such as 2.64 $l.m^{-2}$, besides reducing the number of leaves caused reduction of aerial biomass and proportion of live aerial/total biomass. The exposure time was determinant to the impact of oil on plants, pointing out the high sensitivity of this species.

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