Tolerance of cerrado baru tree (*Dipteryx alata*) submitted to different doses of glyphosate[1]

Amanda Munielly Alves Bernardes[2], Thamires Marques Moura[2], Estenio Moreira Alves[2], Maristela Aparecida Dias[2], Vânia Sardinha dos Santos Diniz[2], Darliane de Castro Santos[3]*

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ABSTRACT

Baru tree (*Dipteryx alata* Vogel.) is a been an alternative to establish an Integration Crop-Livestock-Forestry Systems (ICLFS), combining native species preservation with sustainable food production. However, the management procedure of herbicides for weed control is one of the major limiting factors to include tree components within ICLFS. Thus, the study evaluated the tolerance of *D. alata* submitted to different concentrations of glyphosate on the plants, to make the introduction of this forest species in ICLFS. The treatments comprised different doses glyphosate: 0.0 (control); 960; 1920; 2880 and 3840 g a.e ha^{-1}. Shoot height, stem diameter, shoot dry weight, root dry weight and total dry weight, as well as Dickson Quality Index and phytotoxicity were assessed. The phytotoxicity in plants varies according to the applied doses of glyphosate, with progressive yellowing in the leaves up to well-developed necrosis and leaf senescence in the highest doses. All doses tested reduced the biometric and qualitative attributes of *D. alata* seedlings, demonstrating that no treatment was selective, although the plants are tolerant at dose 960 g a.e ha^{-1} tested. Increases in doses of glyphosate do not cause the death of seedlings *D. alata* that shows a great potential to compose ICLFS.

Keywords: herbicide; native; integration crop-livestock-forestry.

INTRODUCTION

The modernization of Brazilian agriculture from the 1950s has benefited the production of commodities. This system has been characterized by intensive monoculture practice and negative environmental impacts since the expansion of agriculture in Brazilian is also known by the possession of areas upon local deforestation (Barboza *et al.*, 2012). Study published by Strassburg *et al.* (2017) shows that, if the current pace of deforestation is maintained, the Cerrado may suffer the greatest plant extinction in history until 2050, as in the last 30 years, the advance of extensive cattle raising, agriculture with annual crops, and the opening up of new areas, destroyed much of this domain, which stands out for being the second largest morphoclimatic domain in South America, covering about 22% of the Brazilian territory, in different states in the Midwest of the country (MMA, 2020) , holding rich forest species with great potential to be explored in Integration Crop-Livestock-Forestry Systems (ICLFS).

The ICLFS are appointed as agricultural production systems that seeks to combine the productivity with the mitigation of negative environmental impacts. According to FAO (2011), crop-livestock-forest is an intentional integration that reflects a synergistic relationship between agricultural components, animals and/or trees, resulting in social, economic and environmental recovery, optimizing farmers livelihoods involved with its management.

As the Brazilian Midwest region, occupied by the Cerrado, has great economic importance due to its expressive grain production, responsible for more than 111 million tons in the 2019/2020 crop season, representing 45.3% of the national average (IBGE, 2020) studies that

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[2]Instituto Federal Goiano, Iporá, Goiás, Brazil. amandamuni123@gmail.com; thamires.moura@ifgoiano.edu.br; estenio.moreira@ifgoiano.edu.br; maristela.dias@ifgoiano.edu.br; vania.diniz@ifgoiano.edu.br

[3]Instituto Federal Goiano, Rio Verde, Goiás, Brazil. darliane.castro@ifgoiano.edu.br

*Corresponding author: amandamuni123@gmail.com

enable the use of native species are fundamental, both in terms of species conservation and economic viability. Among these species, baru tree (*Dipteryx alata* Vogel.) stands out by presenting multiple uses and purposes, such as food, timber, medicinal, industrial, landscape, and for degraded areas restoration (Alves *et al.*, 2010).

However, one of the limiting factors to introduce a tree component in the ICLFS is the control of weeds by herbicides, since they are frequently used in crops, but they can cause phytotoxic effects in forest species, especially during the phase of establishment in the field.

As one of the most used molecules in weed control, glyphosate is a non-selective desiccant herbicide that controls a wide number of broadleaf and grassy weeds, both annual and perennial. Thus, this study evaluated the initial tolerance of *D. alata* seedlings submitted to application on them of different doses of herbicide glyphosate, seeking answers to make the introduction of this forest species in ICLFS.

MATERIAL AND METHODS

The experiment was performed in the Iporá municipality, western Goiás, which presents a typical Cerrado vegetation. The climatic classification is semi-humid tropical according to Köppen-Geiger's system, with irregular precipitation and distribution throughout the year (Alves, 2011).

The trial was carried out in a greenhouse located at the Experimental Unit of the Horticulture sector, Instituto Federal Goiano - Campus Iporá. The *D. alata* seedlings were produced from seeds collected from progenies located in areas with protected native species of Cerrado in the municipality of Iporá.

For the seed's extraction procedure, fruits were opened by using a coconut breaker. Subsequently, seeds were sown in polystyrene trays. Then, most of the homogeneous seedlings were transplanted to polypropylene tube type pots, with a volume of 3,780 cm^3 containing Latosol Red Dystrophic- LVdf (Santos *et al.*, 2018).

The experiment was conducted in a completely randomized design, with five doses and six replications, which consisted of glyphosate (Roundup transbord®) doses composed of N-(phosphonometh) glycine and isopropylamine salt 480 g L^{-1} acid equivalent (a.e). The applied doses were 0.0; 960; 1920; 2880 and 3840 g a.e ha^{-1}, based on the product manufacturer's recommendation (360 to 2160 g a.e ha^{-1}) for plant desiccation. It is noteworthy that any dose applied less than the recommended is considered a sub-dose, while doses over the maximum are considered overdoses. Thus, there was no use of any sub-dose in this work, but two overdoses.

The experimental unit consisted of one seedling of baru tree growing in a polypropylene pot. The herbicide was applied by using a pressurized CO_2 sprayer, equipped with a 110-2 double fan type air induction tip (AITTJ60), regulated in constant pressure of 2.5 bar. The plants remained protected from contact with either rainwater or irrigation for 24 hours after application to avoid product washing. The plants were irrigated daily, as the same for weeds removal and full sun exposure.

The herbicide was applied directly to the plants which presented 20 cm high and 5 mm in diameter. At both 32 and 64 days after application (DAA), shoot height (H) was measured (from stem to the apical bud, in centimeters), while the stem diameter (D) was assessed with a digital caliper (stem diameter at 0.5 centimeters above the ground, in millimeters).

The phytotoxicity was observed in the seedlings at 2, 4, 8, 16, 24, 32, 64 DAA, when damages were recorded into pictures and classified by a scale ranging from 0 for normal plants (equal to the control), and 100 for dead plants, according to the EWRC (1964) scale, modified by Frans (1972), as shown in Table 1.

Dry weight was determined at 64 days after application (DAA). For this, plants were partitioned into shoot/root, washed, packed in properly identified paper bags, and placed in an oven with forced air circulation for 72 h at 65 ºC until the weight stabilization. Finally, shoot dry weight (SDW, in g), root dry weight (RDW, in g) and total dry weight (TDW, in g) were assessed.

The seedlings quality was determined by Dickson Quality Index (DQI) (Dickson *et al.*, 1960):

$$DQI = TDW / (H / D) + (SDW / RDW)$$

Where: DQI = Dickson Quality Index; TDW = total dry weight (g); H = height (cm), D = stem diameter (mm); SDW = shoot dry weight (g); and RDW = root dry weight (g).

The normality distribution of the data was verified through the Shapiro-Wilk test. The data were submitted to analysis of variance (ANOVA) by Sisvar software was used to examine the effects of quantitative treatments, with the phytotoxicity data were submitted to Tukey's test ($p \leq 0.05$), and the other results were accomplished by regression analysis and "F" test ($p \leq 0.01$) using Sigmaplot software.

RESULTS AND DISCUSSION

Based on the data, no statistical model was identified to fit plant height and stem diameter variables at any evaluated times. Ferreira *et al.* (2005) reported that the response variation in native trees within the same species is a consequence of genetic variability. This result also demonstrates that is necessary to carry out a test for a longer period to determine the behavior of baru tree (*D. alata*) submitted to different doses of glyphosate, since it is a slow-growing species. In this study therefore the determination coefficients (R^2) for these variables showed low potential for explaining the biological behavior by the equations.

For phytotoxicity assessment, we observed both mild and very mild toxicity symptoms (Figure 1B), with yellowing leaves at 4 DAA. According to Campbell *et al.* (1976) and Cole *et al.* (1983), this yellowing is the result of chloroplast degradation and/or inhibition of chlorophyll biosynthesis.

It was observed that the phytotoxicity in *D. alata* plants varies according to the applied doses of glyphosate ($p < 0.05$) (Table 2), reaching maximum ratings of 13.78 and 20.18% at the 32 DAA, respectively, at doses of 960 and 1920 g a.e ha^{-1}, and 28.4 and 35% at the 16 DAA, respectively, at doses of 2880 and 3840 g a.e ha^{-1}. Study by Costa *et al.* (2009) also demonstrated a significant increase in the symptoms of phytointoxication with increasing doses of simulated glyphosate drift in the initial growth of *Jatropha curcas* (L.) seedlings, observing averages of 21, 38.7 and 50% for doses of 90, 180 and 360 g ha^{-1}, respectively.

Table 1: Visual phytotoxicity scale used to evaluate the effect of the herbicide applied on seedlings *D. alata*

Scale	Phytotoxicity (%)	Phytotoxicity characteristic
1	0.0	Null
2	1 – 3.5	Very light
3	3.6 – 7.0	Light
4	7.1 – 12.5	No reflection on production
5	12.6 – 20.0	Average
6	20.1 – 30.0	Almost strong
7	30.1 – 50.0	Strong
8	50.1 – 99.0	Very strong
9	100	Plant death

In the present study, yellowing symptoms was getting more intense, which led to necrosis and consequently death of buds up to 32 DAA (Figure 1C), when presented start new buds too. Costa *et al.* (2009) at 41 DAA verified that in this period *J. curcas* plants began to show recovery of the normal color of the leaves, with disappearance of the symptoms observed in plants exposed to doses of 11.3, 22.5 and 45 g ha^{-1}, but with emission of non-viable (abnormal) shoots for doses of 90, 180 and 360 g ha^{-1}.

Similar phytotoxicity behavior were verified in a study by Yamashita *et al.* (2009), which the authors observed toxic effects of the glyphosate in both tree *Ceiba pentandra* ((L.) Gaertn) and *Schizolobium parahyba* var. *amazonicum* ((Huber ex Ducke) Barneby). The symptoms were characterized by the progressive yellowing in the leaves in response to glyphosate application, which was also verified in other forest species, such as *Hevea brasiliensis* (Aubl.) (Farias *et al.*, 2012), and eucalyptus (Tuffi Santos *et al.*, 2006).

Despite the symptoms presented, none of the doses tested caused the death of *D. alata* plants after glyphosate application, it was also observed an attenuation of symptoms and new sprouts development (Figure 1D), which indicates the recovery of your growth. Likewise, Machado *et al.* (2013) observed that even at lowest sub-dose of glyphosate (160 g a.e ha^{-1}), symptoms of toxicity, as chlorosis, were observed in candeia (*Plathymenia reticulata* Benth.), sucupira-preta (*Bowdichia virgilioides* Kunth), pau-santo (*Kielmeyera lathrophyton* Saddi) and lobeira (*Solanum lycocarpum* A. St. Hil.) seedlings, which evolved to yellowing and then to necrosis. They also observed that, among surviving plants, new leaves appeared mainly after 21 DAA, and leaves were gradually becoming senescent and replaced during seedlings development.

The shoot dry weight, root dry weight, total dry weight and Dickson Quality Index were significantly adjusted by equations (Figure 2A, 2B, 2C and 2D, respectively), with decrease in such responses in *D. alata* plants as a function of increases in the doses. Glyphosate has primary effects

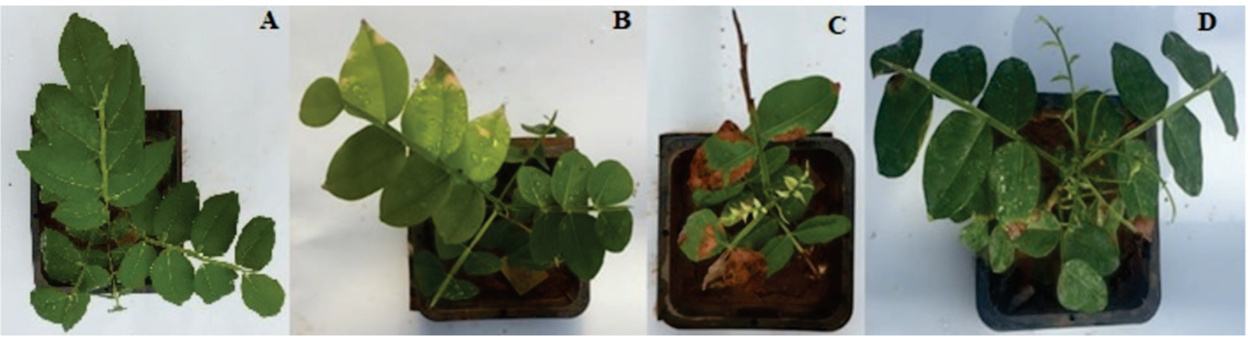

Figure 1: General aspects of seedlings *D. alata* without application (A), with mild and very mild toxicity symptoms at the 4th DAA (B); necrosis symptoms, death of buds and sprouts at the 32th DAA (C); attenuation of symptoms and growth recovery at the 64th DAA (D).

on plants, inhibiting or slowing their growth, due to amino acid biosynthesis pathway inhibition, which reduces its formation and depletes carbon flow and protein synthesis (Gomes *et al.*, 2015). Furthermore, dehydrated leaves and necrosis result in leaf senescence, causing there was a decrease in the shoot and root dry matter.

Study reported by Pereira *et al.* (2015) with *Cedrela odorata* (L.) demonstrated equivalent results. They observed that the shoot dry weight decreased linearly in response to increases in the herbicide doses. Cerveira Junior *et al.* (2020) also observed a significant reduction in the shoot dry weight caused by low doses of glyphosate ($\leq$ 180 g a.e ha^{-1}), while a strong reduction in plant growth (60%) with recommended doses of glyphosate ($\geq$ 720 g a.e ha^{-1}) in eucalyptus (GG100 and I144).

In close agreement with these results, Yamashita *et al.* (2009) observed that glyphosate treatments significantly reduced dry shoot weight of *C. pentandra* and *S. amazonicum* at sub-doses of 180 and 360 g a.e ha^{-1}. In addition, plants of *S. amazonicum* at 28 DAA presented a significant reduction in total dry weight (TDW) of 32% and 38% at doses of 180 and 360 g and ha^{-1} of glyphosate, respectively, as compared to the control. At the same doses of 180 and 360 g a.e ha^{-1}, *C. pentandra* showed a reduction of 16% and 35% in total dry weight, respectively, as compared to the control.

Table 2: Visual phytotoxicity scale used to evaluate the effect of the herbicide applied on seedlings *D. alata*

Doses (g a.e. ha^{-1})	Days After Application (DAA)						
	2	4	8	16	24	32	64
0	0.0a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
960	0.0a	2.16 ab	7.68 ab	6.42 ab	7.15 a	12.42 b	6.86 a
1920	0.0a	4.51 ab	13.63 b	16.80 bc	20.02 b	20.18 c	17.89 b
2880	0.0a	2.00 ab	14.85 b	28.40 cd	22.4 bc	27.98 c	18.85 b
3840	0.0a	3.33 b	18.82 b	35.00 d	29.3 c	30.77 c	25.35 b

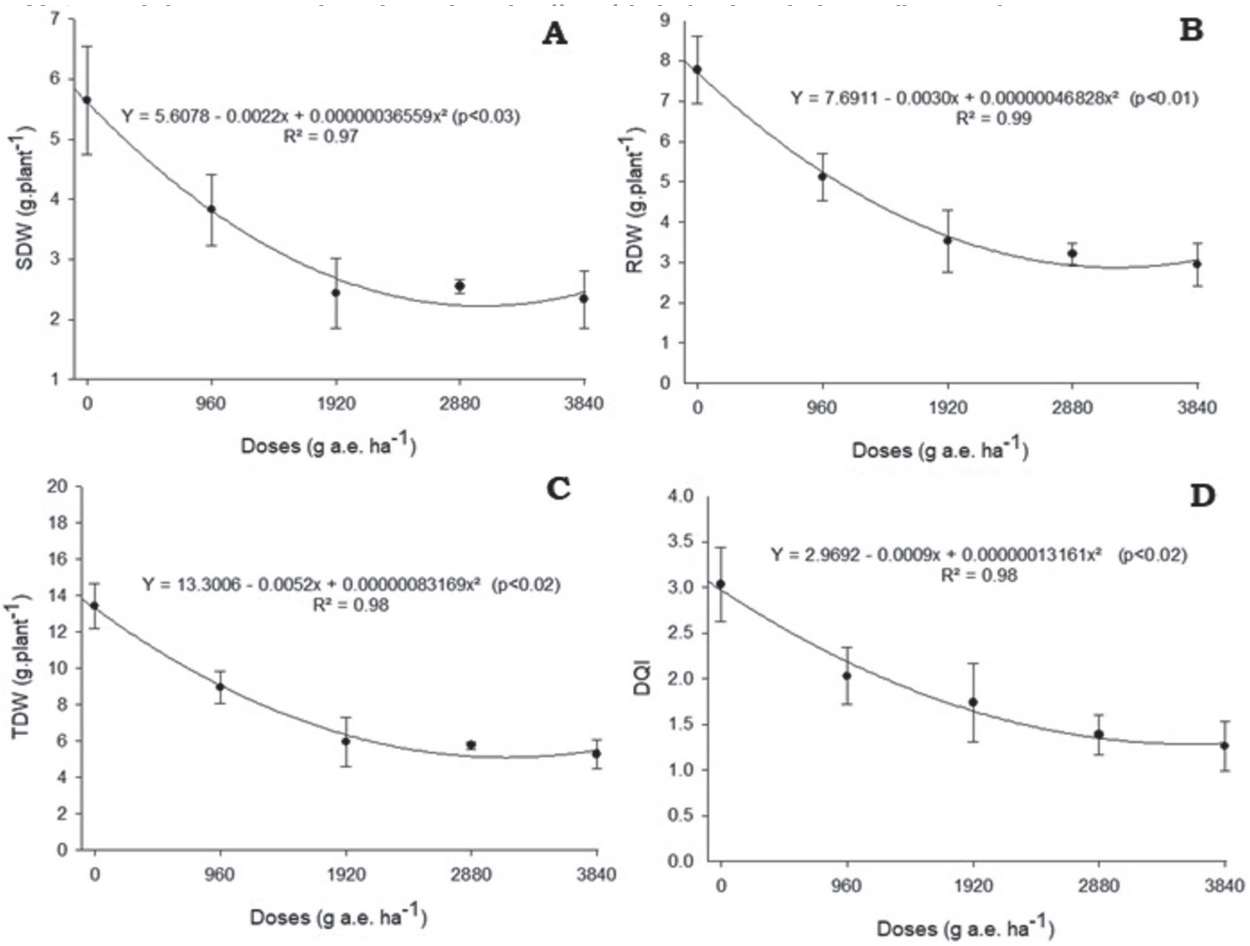

Figure 2: Shoot dry weight (A), root dry weight (B), total dry weight (C) and Dickson Quality Index (D) of *D. alata* seedlings at 64 DAA as a function of different doses of glyphosate.

Christoffoleti *et al.* (2016) define tolerance as an innate characteristic of the species in surviving the application of herbicides at the recommended dose, which would be lethal to other species, without marked changes in their growth and development. Thus, despite all treatments causing symptoms of phytotoxicity and reduction of all variables evaluated in this study, the results demonstrate that *D. alata* plants are tolerant at dose 960 g ae ha^{-1}, as during the conduct of the study the phytotoxicity symptoms of this treatment did not reflect on the general performance of the species, where at 64 DAA the plants no longer differed from the control treatment (Table 2).

The use of several parameters is quite recommended to evaluate the quality of seedlings since isolated indices have not been effective. The seedling quality index proposed by Dickson (DQI) is considered an effective indicator of quality because it takes into account the balance of biomass distribution, weighing several important parameters (Fonseca *et al.*, 2002).

Caldeira *et al.* (2012) and Silva *et al.* (2013) claimed that the higher the DQI, the better is the seedling quality. In this sense, considering 0.20 as a standard minimum value - as recommended by Hunt (1990) - it was observed that *D. alata* seedlings of all evaluated treatments showed a suitable quality to be transferred to the field, with DQI values always above 0.20 (Figure 2D). However, the higher the dosage of glyphosate applied to the seedlings, the lower the DQI averages, evidencing the importance of being warned during application of glyphosate-based pesticides in ICLFS areas with *D. alata* species, avoiding negative effects on its vigor and fitting growth in the field.

Based on the observed response in this study, it is possible to affirm that in case of glyphosate application dose of 3840 g a.e ha^{-1} (equivalent to 8 L ha^{-1}), *D. alata* trees will survive and recover their growth. However, it is necessary to evaluate for a longer period to determine long-term growth behavior of *D. alata* seedlings submitted to glyphosate, an example of Farias *et al.* (2012) that evidenced that the *H. brasiliensis* growth was recovered at the 180 days after herbicide application (345.6 g ha^{-1}), presents tolerance to glyphosate drift.

The introduction of forest species at the beginning of rainy season is essential for the establishment of seedlings in the field since planting at such conditions allows higher growth in height and stem diameter (Melotto *et al.*, 2009). However, this period coincides with the cultivation of annual crops, and the use of herbicides in agrosilvopastoral systems can expose forest species to the risk of drift and or accidental applications.

In this sense, baru tree (*D. alata*) shows a great potential to compose ICLFS with RR technology crop species (corn and soybeans) at the beginning of rainy season, where the glyphosate can be used as a weed management molecule in these crops and will not eliminate *D. alata* plants in consequence of likely drift or accidental application. However, it is important to bear in mind that application on them it reduces growth, being essential the proper management to reduce of accidental applications, allowing a better plant development.

CONCLUSIONS

It is concluded that the use of increasing doses of glyphosate, including overdoses up to 3840 g ha^{-1}, causes a reduction in growth and the appearance of symptoms of phytotoxicity of *D. alata* seedlings, demonstrating that no treatment was selective, however, the plants are tolerant at dose 960 g a.e ha^{-1} tested.

Increases in doses of glyphosate do not cause the death of seedlings *D. alata*, which, in turn, showed growth recovery. These results indicate the potential use of *D. alata* in ICLFS. In addition, the application of glyphosate on seedlings can confer selectivity on *D. alata* plants, being necessary to evaluate the development of plants for a longer period to determine the long-term behavior of seedlings submitted to different doses.

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