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ORIGINAL ARTICLE

Initial growth of tree species under herbicide drift

Crescimento inicial de espécies arbóreas sob deriva de herbicidas

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ABSTRACT: The present study aimed to evaluate the effect of different doses of the herbicides picloram, glyphosate, glyphosate + 2.4-D, and 2.4-D on the survival and growth of the tree species *Croton floribundus* and *Heliocarpus americanus* and *Myrsine coriacea.* The experimental design was completely randomized with four replicates. The tree species were sprayed with 0; 2.5; 5; 10; 20; 40; 60; 75; 90 and 100% of the commercial dose of each herbicide. The percentage of phytotoxicity, chlorophyll content, height, stem diameter, leaf area and aerial dry biomass of the species were evaluated. For *H. americanus*, the herbicides glyphosate, 2.4-D and glyphosate + 2.4-D were phytotoxic. Among the evaluated herbicides, picloram was the most phytotoxic for all species. Glyphosate presented lower phytotoxicity at lower doses for *M. coriacea* compared with *C. floribundus* and *H. americanus*. A negative impact of 2.4-D was verified in all species, even at the lowest doses. The mixture glyphosate + 2.4-D also presented high phytotoxicity, with lower values for the species *M. coriacea*. The results showed that the application of these herbicides should be directed and protected, and even low dosages have deleterious effects on these species.

RESUMO: *O objetivo deste trabalho foi avaliar o efeito de diferentes doses dos herbicidas picloram, glyphosate, glyphosate + 2,4-D e 2,4-D na sobrevivência e no crescimento das espécies arbóreas Croton floribundus, Heliocarpus americanus e Myrsine coriacea. O delineamento experimental foi inteiramente casualizado, com quatro repetições. As espécies arbóreas foram pulverizadas com 0; 2,5; 5; 10; 20; 40; 60; 75; 90 e 100% da dose comercial de cada herbicida. Foram avaliadas a porcentagem de fitotoxicidade, índice de clorofila, altura, diâmetro do caule e a área foliar, assim como a biomassa seca da parte aérea das espécies. Para H. americanus, os herbicidas glyphosate, 2,4-D e glyphosate + 2,4-D foram fitotóxicos. Dentre os herbicidas avaliados o picloram foi o mais fitotóxico para todas as espécies. O glyphosate apresentou menor fitotoxicidade nas menores doses para M. coriacea em relação à C. floribundus e H. americanus. Impacto negativo do 2,4-D foi verificado em todas as espécies mesmo nas menores doses. A mistura glyphosate + 2,4-D também apresentou alta fitotoxicidade sendo observados menores valores para a espécie M. coriacea. Os resultados permitem concluir que aplicações desses herbicidas devem ser dirigidas e com proteção, sendo que mesmo baixas doses apresentam efeito deletério para essas espécies.*

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1 Introduction

Semideciduous seasonal forests are rich in woody species (Santos & Kinoshita, 2003) but have experienced accelerated fragmentation processes because of intensive disturbances that compromise the maintenance of diversity (Rodrigues & Leitão-Filho 2001). However, studies on the ecological restoration in these formations are scarce (Souza & Batista, 2004).

One of the factors that can negatively influence the growth and development of tree species in restoration areas is the intensive use of herbicides in agricultural areas. The use of chemicals in weed control is common in intensive agricultural and forestry crops, which suffer significant losses in productivity because of interference caused by weeds that compete for essential resources such as water, light and nutrients or by possible allelopathic effects (Vivian et al., 2013; Hill et al., 2007). Drift in restoration areas induced by directed herbicide application is an important factor that negatively affects the ecological restoration of these areas.

Herbicide drift is a problem in many producing area, especially when farmers apply herbicide under environmental conditions that favor volatilization and redeposition, which damages non-target crops in addition to reducing weed control effectiveness (Tuffi Santos et al., 2007). Potential injuries to crops because of herbicide deposition on "non-target" areas has led to several studies on the use of different herbicide dosages, particularly in crops where the applications are performed with aerial equipment like cotton, soybean and wheat (Deeds et al., 2006; Sciumbato et al., 2004), which can affect adjacent ecological restoration areas.

The use of glyphosate as a desiccant or herbicides that mimic auxin, such as 2.4-D and picloram, in pasture fields is common in agricultural areas (Campos et al. 2013). In addition, genes for 2.4-D resistance are being incorporated into soybean plants, likely implying a greater usage of these herbicides in the field, which may produce greater volatilization and drift capacity (Behrens et al., 2007). Therefore, it is more likely that these herbicides will be found in adjacent areas, and adverse effects on susceptible species will increase (Marple et al., 2008; Marple et al., 2007; Kruger et al., 2012). Symptoms of chlorosis, necrosis and twisting may be observed on the leaves of plants susceptible to 2.4-D and picloram (Johnson et al., 2012). Chlorosis, necrosis, multiple budding (because of the death of apical buds), winding, reddening and narrowing of the leaf blade are symptoms exhibited by species susceptible to glyphosate (Tuffi Santos et al., 2006; Gravena et al., 2009), with chlorosis as a result of the degeneration of chloroplasts and inhibition of chlorophyll (Tuffi Santos et al., 2007).

The selectivity of herbicides used in *Eucalyptus* spp. fields on the growth of *Myracrodruon urundeuva* (aroeira) was differentiated according to the active substance. The herbicide that showed the highest phytotoxicity and best inhibited the development of *M. urundeuva* was glyphosate. The herbicides haloxyfop-methyl, sulfentrazone and oxyfluorfen did not compromise the development of seedlings (Duarte et al., 2006).

The present study aimed to determine the effects of glyphosate, glyphosate + 2.4-D, 2.4-D and picloram on the development of *Croton floribundus, Heliocarpus americanus* and *Myrsine coriacea*.

2 Material and Methods

The experiments were performed in greenhouse, and the experimental design for each herbicide was completely randomized and included four replicates. Each experimental unit consisted of a tree species seedling, with *Croton floribundus, Heliocarpus americanus* and *Myriaceae coriacea* seedlings acquired from nurseries and transplanted into 5 L pots filled with substrate composed of soil and bio-stabilized *Pinus* bark in a 3:1 ratio. At the time of transplantation, the plant height was 20 cm for *C. floribundus*, 50 cm for *H. americanus* and 40 cm for *M. coriaceae*. The plants remained in a greenhouse with automatic irrigation for 15 days prior to herbicide application.

The tree species were sprayed with 0, 2.5; 5; 10; 20; 40; 60; 75; 90 and 100% of the commercial dose of 2.4-D (2.4-D Nortox (670 g L^{-1}) - 1000 g i.a. ha^{-1}), glyphosate (Roundup (360 g L^{-1}) - 760 g i.a ha^{-1}), glyphosate + 2.4-D (2.4-D Nortox (670 g L^{-1})/ Roundup (360 g L^{-1}) - 760 g i.a ha^{-1} + 1000 g i.a ha^{-1}), and picloram (Padron (240 g L^{-1}- 2% v/v). The herbicides were applied by pressurized CO_2 spray at a constant pressure of 35 psi with an application beam with spray nozzles of the 110.03 flat-fan type. The spray volume was 200 L ha^{-1}. The relative humidity (70%) and room temperature (25-28 °C) throughout the product applications were monitored by a weather station. The plants were allocated in greenhouse and maintained with adequate moisture. The herbicide effect was evaluated at 15; 30; 60; 90 and 120 days after application (DAA) according to percentage score scale, where zero corresponds to no injury and 100 corresponds to plant death (Alam, 1974). The height of the plants was determined at 30; 60 and 120 DAA. The chlorophyll content was measured at 15 and 120 DAA with a chlorophyll meter (ChlorofiLOG - FALKER), and the stem diameter at 3 cm from the ground, leaf area (non-destructive method with a LiCor 3000 leaf area meter), and aerial dry biomass cut close to the ground were determined at 120 DAA. Dry mass was obtained by placing the plants in a forced-air circulation oven at 65 ºC and drying to a constant weight.

The data were subjected to an analysis of variance (ANOVA) and regression. The regression curves were fitted with the SIGMAPLOT software 13.0.0.83. For certain analyses, the means were compared by Tukey's test at 5% probability.

3 Results and Discussion

The percentage of phytotoxicity caused by the herbicides on the species *H. americanus* can be observed in Figure 1. The phytotoxicity reached maximum levels at most doses at 30 DAA, and after this period, there was a slight decrease in symptoms. Glyphosate at the commercial dosage (100% of the dose) caused changes in phytotoxicity from 51.1% at 15 DAA to 67.6% at 30 DAA, with significant chlorosis and necrosis. In the evaluation conducted at 120 DAA, as the herbicide dosage was increased, a greater effect was detected on the plants. At 10 and 20% of the commercial dosage of glyphosate, the observed phytotoxicity was 17.8 and 31.0%, respectively (Figure 1A). The predominant use of glyphosate for resistant crops presents significant opportunities for drift (Johnson et al., 2006), and it is one of the most widely used herbicides globally (Ghisi & Cestari, 2013).

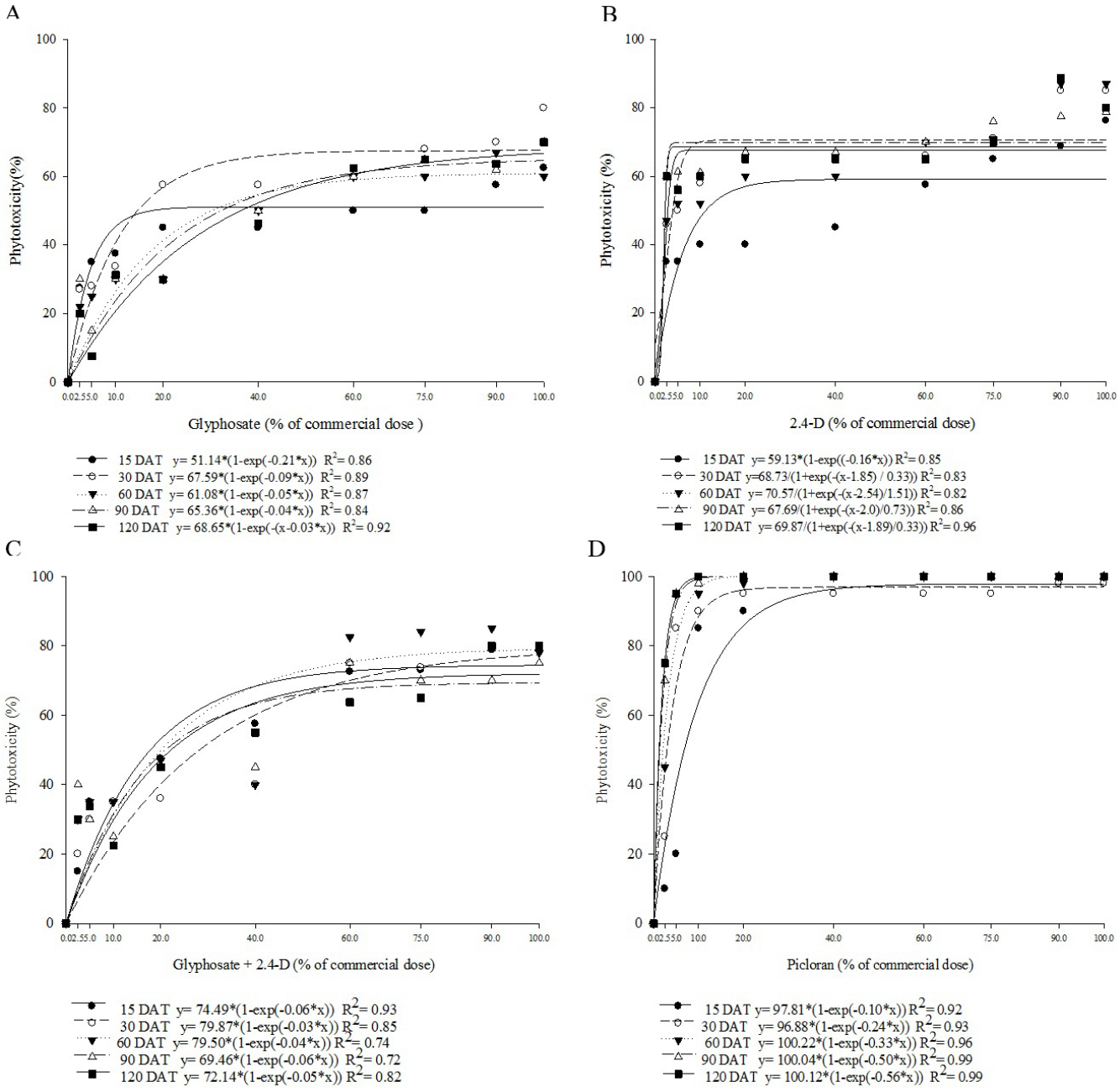

Figure 1. Percentage of phytotoxicity of different doses of glyphosate (A), 2.4-D (B), glyphosate + 2.4-D (C) and picloram (D) to *Heliocarpus americanus* evaluated at 15; 30; 60; 90 and 120 days after transplantation (DAT).

Figura 1. Porcentagem de fitotoxicidade de diferentes doses de glyphosate (A), 2,4D (B), glyphosate + 2,4D (C) e picloran (D) à *Heliocarpus americanus* avaliada aos 15; 30; 60; 90 e 120 dias após o transplante (DAT).

Regarding the use of 2.4-D, rapid evolution of the symptoms occurred even at smaller doses compared with that of glyphosate (Figure 1B). In dosages above 10%, an effect similar to the effect at 100% can be observed, which demonstrates the suppressive effect of this herbicide species, even at low dosages. Phytotoxicity was maintained at 120 DAA even at the lowest dosages. At a 10% dosage, phytotoxicity was 69.9%, which is a similar value to that at the 100% dosage of glyphosate. At each dosage, classic symptoms of this herbicide were observed, such as stem and leaf petiole epinasty and leaf blade cracking and deformation.

The herbicide 2.4-D caused high toxicity to sensitive crops with intensive use, particularly in no-tillage system in Brazil (Costa et al., 2014). With the mixture glyphosate + 2.4-D, increased phytotoxicity was observed at higher dosages (Figure 1C). The greatest damage to the plants occurred at 60 DAA (78%), with a slight decrease in phytotoxicity at 90 and 120 DAA (approximately 70%). In crop management operations, 2.4-D is commonly associated with glyphosate to

control a higher number of weeds, particularly tolerant species or biotypes resistant to glyphosate.

Plant intoxication by glyphosate is common in coffee crops and characterized by morphological alterations and similar symptoms to that of nutritional disorders, such as nitrogen, boron, iron and zinc deficiency (França et al., 2010). However, limited information is available on the effect of the drift of these products in tree species. For *Schizolobium amazonicum* and *Ceiba pentandra*, the dry weight and root length was reduced by the action of the herbicides glyphosate and 2.4-D when applied separately or together. Despite the tolerance of both species to low doses of glyphosate, both herbicides caused significant damage, suggesting directed jet application to avoid losses in plant development (Yamashita et al., 2009).

Picloram showed the highest phytotoxicity values among the evaluated herbicides (Figure 1D). *H. americanus* showed extreme phytotoxicity to this herbicide, with minimum doses of 2.5% resulting in 75% phytotoxicity at 120 DAA. Picloram caused high phytotoxicity even at baseline at 15 DAA, with phytotoxicity of 61.8% at 10% of the commercial dosage, and caused plant death in treatments involving dosages of 40% the commercial dosage.

Regarding plant height, the evaluated dosages of the herbicide glyphosate negatively interfered with plant growth (Table 1). At 30 DAA, a 9.38 cm difference in height was observed in the control plants and plants subjected to a higher dosage. The difference was slightly lower at 120 DAA, but the impact was still observed at the commercial dosage. For glyphosate + 2.4-D and 2.4-D, this difference in height was more pronounced at 120 DAA, although significant differences were observed in all evaluation periods. Picloram showed little difference between the doses at 30 DAA; however, this difference was more pronounced at 120 DAA, especially between the highest doses and control. These results highlight the negative impact of the herbicides on the continued growth of seedlings in general (Table 1). Picloran caused a negative impact on stem diameter of the *H. americanus* seedlings, especially at the highest doses. At smaller doses, the values changed, although this result can be attributed to the variability in thickening among the plants, which is typical of tree species. Although the diameter of the plants treated with picloram was recorded, the seedlings were irreversibly necrotic.

With respect to the aerial part biomass, leaf area and chlorophyll content, the application of glyphosate caused significant differences in biomass at the highest dose and in the leaf area at 75% of the commercial dosage (Table 1). The height and diameter indicated that the species is more tolerant to glyphosate. The mixture glyphosate + 2.4-D caused differences in the production of aerial part biomass, with a significant decrease at 60% of the commercial dose. The herbicide 2.4-D negatively affected the aerial part biomass and leaf areas, especially at the highest doses. Picloram promoted the greatest impact on this species, which confirms the phytotoxicity results. Plant death occurred at 5% of the commercial dosage, and drying of the stem and absence of leaf area and chlorophyll were observed at 120 DAT (Table 1).

The phytotoxic effects of the herbicides on the species *C. floribundus* are shown in Figure 2. Compared with the species *H. americanus*, plant recovery occurred along with development, and increasing phytotoxicity is observed throughout the evaluations for *C. floribundus*. High species control (above 80%) was observed with glyphosate at dosages above 20% (Figure 2A), which indicates the greater susceptibility of this species to glyphosate compared with that of *H. americanus*.

Effect of glyphosate on *Plathymenia reticulata, Bowdichia virgilioides, Kielmeyera lathrophyton* and *Solanum lycocarpum* were chlorosis and leaf necrosis, and *S. lycocarpum* was most affected by this herbicide, with death occurring at the highest doses of glyphosate. However, normal seedling development occurred in *P. reticulata, B. virgilioides* and *K. lathrophyton*, which indicates that these species are tolerant to glyphosate and shows the differential response of tree species to the herbicides as found in the present study (Santos et al., 2003).

Regarding the effect of 2.4-D, damage, including stem epinasty and blade deformity, increased with species development. Phytotoxicity was high (75.5%) at 10% of the commercial dosage (Figure 2B). These low dosages and increased effects on plants indicate the high susceptibility of these tree species to these herbicides.

The mixture glyphosate + 2.4D showed slightly lower phytotoxicity symptoms compared with the herbicides applied alone. At 120 DAA, values of 60.5% were recorded for 10% of the commercial dosage and approximately 70% at 20% the commercial dosage (Figure 2C). In a study conducted by Yamashita et al. (2009), from the seventh day of glyphosate + 2.4-D amine (180 + 335 g ha^{-1}) application, wilting and yellowing of the apex leaves of *Ceiba pentandra* was observed.

The use of the glyphosate and 2.4-D mixture is justified in agricultural areas because it provided better control of various weeds, such as *Commelina villosa* (Campos et al., 2013), *Commelina benghalensis* (Maciel et al., 2011) and *Conyza canadensis* and *Conyza bonariensis*, relative to the products applied alone (Procópio et al., 2006). The results show that the deleterious effects on the tree species require careful application of these herbicides.

Among the studied herbicides, picloram, which is widely used to control eudicotyledons in pastures, was the least selective. According to the regression equation, 20% of the commercial dose resulted in 96.4% phytotoxicity, which was maintained at other doses. The plants presented stem epinasty and wringing even at 10% of the commercial dose, which culminated in standstill growth and high phytotoxicity (Figure 2D). Picloram is a hormonal herbicide, and small doses cause great effects. Experiments with coffee in soil with picloram residue showed that over time (evaluations conducted at 60 and 120 DAP), recovery of the plants intoxicated by the herbicide did not occur (D'Antonino et al., 2012).

The effect of the tested herbicides on the height of *C. floribundus* are presented in Table 2. Glyphosate affected the height, and the difference in height between the control treatment and commercial dose was 11.50 cm. With the mixture glyphosate + 2.4-D, significant differences were only observed at 120 DAA at the commercial dose, with a 50% decrease compared with that of the control. Herbicidal effect was not detected for sub-doses, which was also observed in other studies (Brancalion et al., 2009).

Table 1. Shoot biomass, leaf area, height, chlorophyll content and stem diameter of *Heliocarpus americanus* under different dosages of the herbicides glyphosate, glyphosate + 2.4- D; 2.4-D, and picloram.

Tabela 1. Biomassa da parte aérea, área foliar, altura, conteúdo de clorofila e diâmetro de colmo de *Heliocarpus americanus* submetidos em diferentes doses de herbicida glyphosate, glyphosate +2,4-D, 2,4-D e picloram.

% of the commercial dose	Shoot biomass (g)	Leaf area (cm^2)	Height (cm)			Chlorophyll content		Stem diameter (cm)
Glyphosate			**30 DAT**	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	20.19 a	677.02 a	64.88 a	68.75 a	79.13 a	38.75 a	36.75 a	0.95 a
2.5	16.82 a	603.73 ab	62.50 b	68.25 a	78.25 a	37.00 a	38.50 a	0.80 abc
5	19.08 a	535.52 ab	59.25 c	67.00 a	78.13 a	38.50 a	38.00 a	0.90 ab
10	18.00 a	594.55 ab	63.88 a	66.50 ab	77.00 ab	37.50 a	35.50 a	0.80 abc
20	20.31 a	673.90 a	57.75 cd	66.00 ab	75.63 b	40.00 a	35.00 a	0.82 ab
40	18.42 a	573.92 ab	59.50 c	63.00 bc	74.75 b	31.25 a	31.75 a	0.75 bc
60	15.25 a	494.45 ab	59.75 c	62.38 cd	76.10 b	35.75 a	40.25 a	0.67 c
75	17.48 a	382.93 b	59.00 c	63.00 bc	74.50 b	33.00 a	32.25 a	0.87 ab
90	13.48 ab	364.63 b	56.50 d	60.25 d	74.00 b	33.25 a	35.50 a	0.82 ab
100	12.00 b	377.95 b	55.50 d	59.13 d	70.00 c	33.75 a	34.50 a	0.82 ab
CV (%)	31.75	20.08	10.92	2.58	12.22	20.65	13.88	9.40
Glyphosate + 2.4D	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	24.28 a	627.56 a	70.25 a	83.75 a	92.25 a	46.00 a	33.50 a	1.07 a
2.5	17.16 a	628.85 a	65.75 bc	78.50 b	86.00 c	43.75 a	34.50 a	0.95 ab
5	19.09 a	551.32 a	71.25 a	75.00 bc	90.25 b	41.00 a	34.25 a	1.05 a
10	19.97 a	699.67 a	69.25 ab	71.50 c	84.25 d	39.50 a	30.75 a	1.00 a
20	15.19 ab	655.45 a	63.00 cd	74.50 c	85.00 cd	38.50 a	35.75 a	1.00 a
40	22.41 a	576.05 a	62.75 cd	74.50 c	81.50 e	39.50 a	29.00 a	0.87 b
60	11.90 b	634.34 a	61.75 cd	64.75 d	66.75 f	26.75 b	35.00 a	0.90 ab
75	13.31 b	621.45 a	61.50 d	63.75 d	65.50 f	31.50 b	34.00 a	0.82 cd
90	6.23 bc	453.49 a	57.25 e	57.75 e	59.00 g	25.00 b	35.00 a	0.75 d
100	9.64 bc	491.87 a	52.50 f	55.25 e	60.00 g	26.25 b	36.25 a	0.75 d
CV (%)	30.71	20.09	2.62	2.08	6.72	16.65	12.45	8.27
2.4-D	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	19.79 a	486.77 a	73.00 a	84.00 a	89.00 a	34.00 a	37.50 a	0.98 a
2.5	11.38 bc	314.43 b	65.50 bc	76.50 b	82.25 b	38. 50 a	34.50 a	0.97 a
5	11.50 bc	304.93 b	57.00 d	72.50 c	71.50 e	38.75 a	35.50 a	0.85 b
10	13.00 bc	416.99 a	57.25 d	77.25 b	74.00 de	41.25 a	41.25 a	0.87 b
20	11.75 bc	263.90 b	65.50 bc	72.75 b	80.75 d	42.25 a	35.50 a	1.02 a
40	11.25 bc	357.61 ab	66.50 b	61.00 f	82.25 b	41.25 a	36.75 a	1.07 a
60	14.50 ab	489.66 a	65.75 bc	72.50 c	85.00 b	40.50 a	35.75 a	0.87 b
75	11.50 bc	352.24 ab	62.75 c	72.75 c	82.25 b	39.75 a	36.00 a	0.81 b
90	7.75 c	192.00 bc	63.00 c	64.50 e	65.75 f	38.25 a	28.00 a	0.80 b
100	7.50 c	126.00 c	53.00 e	59.25 f	63.25 f	36.00 a	25.75 a	0.80 b
CV (%)	20.22	19.47	2.17	2.77	11.04	13.86	26.19	9.10
Picloram	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	7.65 a	155.25 a	53.88 a	57.50 a	60.50 a	41.75 a	25.50 a	1.00 a
2.5	3.55 b	71.00 b	57.25 a	58.50 a	60.75 a	41.25 ab	28.00 a	0.80 ab
5	3.00 b	0.00 c	49.50 a	53.75 a	40.25 b	25.50 bc	0.00 b	0.60 bc
10	2.00 c	0.00 c	46.38 a	52.25 a	41.50 b	16.50 cd	0.00 b	0.60 bc
20	2.00 c	0.00 c	46.75 a	50.25 a	40.00 b	9.00 de	0.00 b	0.63 bc
40	0.00 c	0.00 c	53.00 a	49.75 a	42.25 b	0.00 e	0.00 b	0.68 bc
60	0.00 c	0.00 c	46.00 a	49.00 a	41.50 b	0.00 e	0.00 b	0.63 bc
75	0.00 c	0.00 c	44.75 a	48.25 a	41.00 b	0.00 e	0.00 b	0.55 c
90	0.00 c	0.00 c	48.00 a	48.75 a	43.00 b	0.00 e	0.00 b	0.58 bc
100	0.00 c	0.00 c	45.25 a	48.75 a	41.25 b	0.00 e	0.00 b	0.73 bc
CV (%)	59.33	61.33	12.52	13.50	14.62	50.13	14.88	14.94

Means followed by the same letter in the column do not differ according to Tukey's test at 5%.

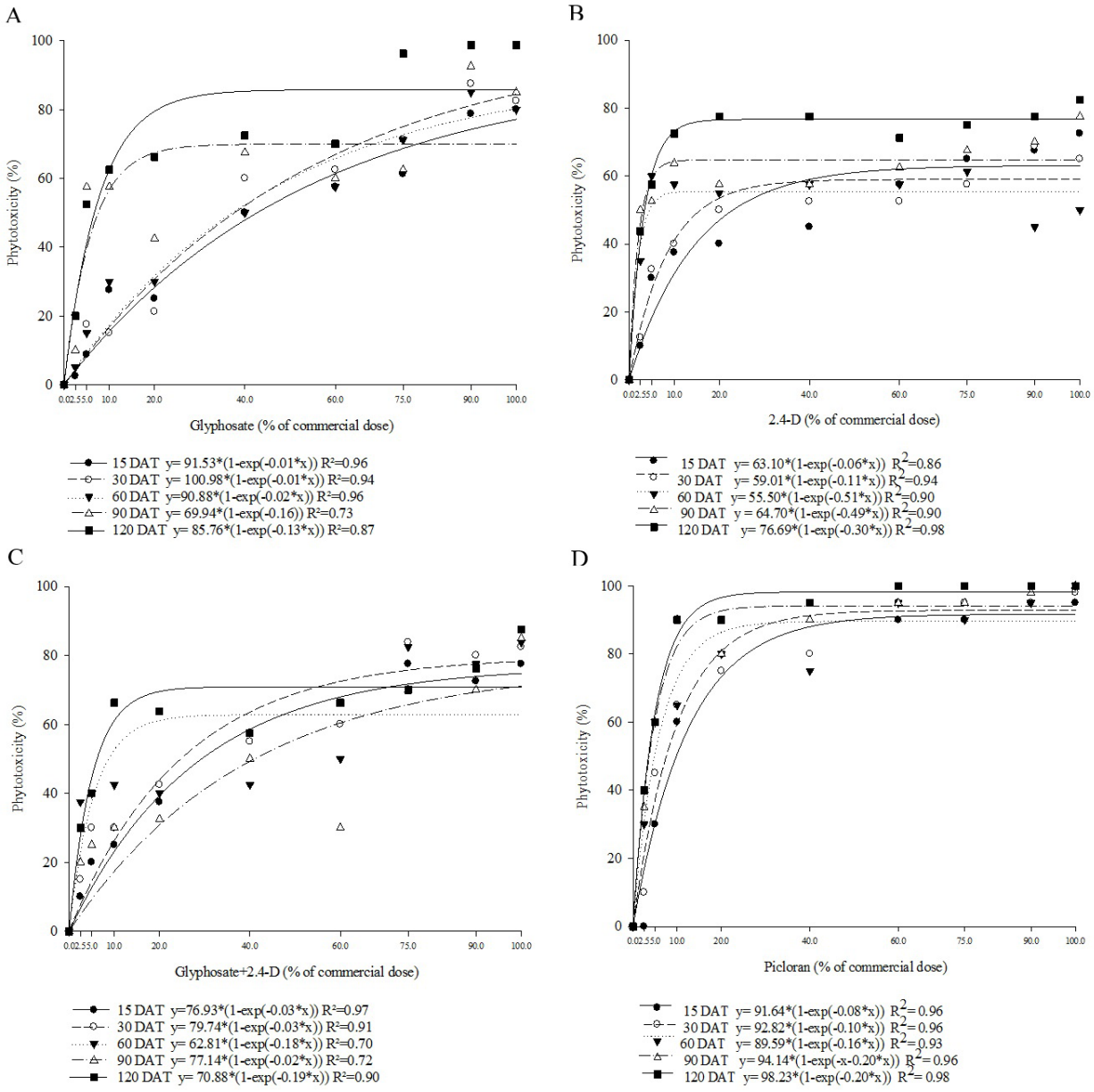

Figure 2. Percentage of phytotoxicity of different doses of glyphosate (A), 2.4-D (B), glyphosate + 2.4-D (C) and picloram (D) to *Croton floribundus* at 15; 30; 60, 90 and 120 days after transplantation (DAT).

Figura 2. Porcentagem de fitotoxicidade de diferentes doses de glyphosate (A), 2,4-D (B), glyphosate+2,4-D (C) e picloran (D) à *Croton floribundus*, avaliada aos 15, 30, 60, 90 e 120 dias após o transplante (DAT).

The isolated use of 2.4-D also affected seedling growth, particularly at commercial doses. Sub-doses of picloram also had an effect on growth. At 120 DAA, the plants subjected to the commercial dose were 25 cm smaller than those exposed to the control, and the stem was necrotic. At 120 DAA, a decreasing trend in stem diameter was observed at the highest doses of the herbicides except for picloram, which showed a more significant effect than the other herbicides, even at intermediate doses (Table 2).

The application of glyphosate and glyphosate + 2.4-D caused a decreasing trend of aerial dry biomass and leaf area with higher doses; however, a direct effect was not observed at the lower doses (Table 2). Brancalion et al. (2009) also did not observe a proportional relationship between the decrease of leaf dry matter weight of *S. multijuga*, *G. ulmifolia* and *C. urucurana* and increases of herbicide dosage. For picloram, this relationship between dose and biomass reduction was more significant, 2.4-D showed significant differences in biomass only at the commercial dose, which can be explained by the herbicidal effect on plant height and stem diameter (Table 2). Effects on chlorophyll content were observed at the highest herbicide doses and are reflected in tissue necrosis.

The herbicidal effect on *M. coriacea* can be observed in Figure 3. The phytotoxicity values obtained for the different evaluation periods show that the dosage effect was permanent, and plant recovery did not occur among the evaluations for each studied dosage. Phytotoxicity evolution of the seedlings was observed with increased doses of glyphosate throughout

Table 2. Shoot biomass, leaf area, height, chlorophyll content and stem diameter of *C. floribundus* under different doses of the herbicides glyphosate, glyphosate + 2.4-D, 2.4-D, and picloram.

Tabela 2. Biomassa da parte aérea, área foliar, altura, índice de clorofila e diâmetro do caule de *C. floribundus* sob diferentes doses dos herbicidas glyphosate, glyphosate+2,4-D, 2,4-D e picloran.

% of the commercial dose	Shoot biomass (g)	Leaf area (cm^2)	Height (cm)			Chlorophyll content		Stem diameter (cm)
Glyphosate			**30 DAT**	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	8.57 a	677.50 a	33.00 a	37.75 a	39.50 a	56.25 a	56.25 a	0.70 a
2.5	8.20 a	566.00 ab	27.38 a	31.38 a	35.75 a	41.50 a	41.50 a	0.55 ab
5	8.91 a	575.00 ab	34.50 a	36.00 a	41.00 a	46.50 a	46.50 a	0.70 a
10	4.98 b	380.25 bc	28.25 a	33.25 a	38.50 a	41.75 a	41.75 a	0.58 ab
20	5.24 ab	118.75 cd	31.00 a	34.50 a	35.75 a	47.00 a	47.00 a	0.78 a
40	2.45 b	62.48 d	32.50 a	33.00 a	35.25 ab	37.50 a	37.50 ab	0.45 b
60	3.48 b	182.00 cd	31.00 a	28.50 ab	30.15 b	40.25 a	40.25 a	0.62 ab
75	1.22 c	34.00 d	23.50 ab	28.75 ab	30.25 b	22.75 b	22.75 bc	0.40 b
90	0.15 c	7.50 d	25.00 ab	29.00 ab	29.00 b	7.50 b	7.50 d	0.13 c
100	0.95 c	2.50 d	18.75 b	17.00 b	18.00 bc	11.25 b	11.25 cd	0.38 bc
CV (%)	39.04	53.43	19.01	16.54	17.48	32.78	32.21	20.11
Glyphosate + 2,4D	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	8.58 a	708.00 a	32.00 a	35.00 a	45.75 a	44.75 a	45.75 a	0.75 a
2.5	6.90 ab	592.25 a	31.50 a	34.00 a	39.75 a	50.75 a	49.50 a	0.65 a
5	9.50 a	492.75 a	31.50 a	31.75 a	45.75 a	52.00 a	51.75 a	0.88 a
10	4.77 ab	304.75 ab	32.75 a	36.00 a	43.00 a	51.50 a	47.00 a	0.60 ab
20	8.07 a	574.75 a	33.75 a	33.75 a	46.50 a	52.75 a	57.25 a	0.75 a
40	4.63 ab	291.00 ab	25.25 a	31.75 a	41.50 a	42.75 a	37.00 a	0.58 ab
60	5.75 ab	317.75 ab	28.00 a	31.75 a	41.50 a	52.75 a	49.50 a	0.58 ab
75	7.08 a	335.50 ab	26.50 a	27.50 a	43.67 a	29.25 ab	26.75 ab	0.60 ab
90	4.75 ab	394.00 ab	25.50 a	26.25 a	41.50 a	37.00 a	28.25 ab	0.58 ab
100	2.03 b	64.25 b	24.00 a	21.50 a	22.75 b	31.50 a	14.50 b	0.48 b
CV (%)	43.16	48.04	17.45	19.83	16.30	22.78	39.63	25.04
2,4-D	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	8.70 a	326.25 a	30.25 b	31.25 a	39.25 a	54.00 a	48.50 a	0.83 ab
2.5	7.58 ab	174.00 a	33.50 a	35.75 a	44.25 a	47.75 ab	42.50 a	0.68 ab
5	5.48 ab	252.25 a	39.25 a	31.25 a	45.00 a	52.00 a	46.25 a	0.85 a
10	6.05 ab	161.50 a	32.00 ab	31.50 a	39.25 a	47.75 ab	30.75 a	0.75 a
20	7.28 ab	147.50 a	34.75 a	28.00 a	35.50 ab	48.00 ab	44.75 a	0.78 a
40	5.48 ab	153.00 a	28.50 bc	26.50 ab	32.50 ab	45.50 ab	43.75 a	0.70 ab
60	5.25 ab	289.75 a	29.75 bc	25.50 ab	37.00 ab	48.25 ab	46.00 a	0.78 a
75	4.95 ab	182.25 a	26.25 bc	23.00 b	34.00 ab	40.75 ab	40.00 a	0.73 a
90	5.25 ab	384.00 a	27.50 bc	26.50 ab	36.25 ab	40.50 ab	47.25 a	0.88 a
100	4.00 b	259.50 a	22.00 c	19.33 b	26.67 b	34.50 b	46.25 a	0.50 b
CV (%)	27.00	55.81	14.34	16.59	15.50	15.62	21.70	18.74
Picloram	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	4.10 a	196.25 a	26.13 a	27.25 ab	30.38 a	56.25 a	35.75 a	0.53 a
2.5	4.63 a	193.25 a	27.20 a	29.50 a	33.00 a	56.75 a	33.25 a	0.30 b
5	3.48 a	160.00 a	24.20 ab	26.25 ab	28.00 ab	52.00 a	27.75 a	0.23 bc
10	1.25 b	51.75 b	25.28 ab	27.25 ab	19.75 ab	46.00 a	36.25 a	0.18 bc
20	1.00 b	17.84 b	24.50 ab	25.75 ab	15.75 ab	44.25 a	15.50 b	0.13 bc
40	0.00 b	0.00 b	22.98 ab	23.00 b	18.50 ab	30.75 ab	0.00 c	0.10 c
60	0.00 b	0.00 b	22.25 ab	23.25 b	18.00 ab	15.50 b	0.00 c	0.10 c
75	0.00 b	0.00 b	23.50 ab	23.50 b	14.50 bc	13.25 b	0.00 c	0.10 c
90	0.00 b	0.00 b	22.18 ab	23.00 b	7.50 c	10.25 b	0.00 c	0.10 c
100	0.00 b	0.00 b	21.33 b	22.00 b	5.00 c	18.25 b	0.00 c	0.10 c
CV (%)	55.65	62.39	10.07	9.76	38.92	44.36	21.01	32.73

Means followed by the same letter in the column do not differ according to Tukey's test at a 5% level of significance.

the evaluations, and at 120 DAA, the commercial dose caused phytotoxicity greater than 70% (Figure 3A).

The herbicide 2.4-D had a more pronounced phytotoxic effect compared with glyphosate, especially at lower doses (Figure 3B). Phytotoxicity values of approximately 75% were observed at 10% of the commercial dose at 120 DAA, whereas with glyphosate, phytotoxicity at the same dose was approximately 20%. In addition, plant recovery did not occur throughout the evaluations. The mixture glyphosate + 2.4-D showed phytotoxicity evolution until 90 DAA, with 61.3% phytotoxicity after 5.0% of the commercial dose. However, during the evaluation at 120 DAA, slight plant recovery was observed, and phytotoxicity was near 50% at 20% of the commercial dose (Figure 3C).

Compared with the other evaluated species, picloram showed a slower effect on *M. coriácea* at lower doses. At 15 and 30 DAA, phytotoxicity was greater with increased herbicide doses, and the phytotoxicity evolution was also verified in the other evaluation periods, with phytotoxicity above 80% at 20% of the commercial dose at 120 DAA (Figure 3D).

The herbicides only had an effect on *M. coriacea* seedling height at 120 DAA with the highest dose; thus, the herbicides had less of a negative impact on this species compared with the other observed species. Stem diameter was reduced only in the treatments with glyphosate + 2.4-D at 120 DAA, and a smaller diameter was observed with the highest dose (Table 3). Machado et al. (2013) did not observe differences in height and stem diameter of the native species *Bowdichia*

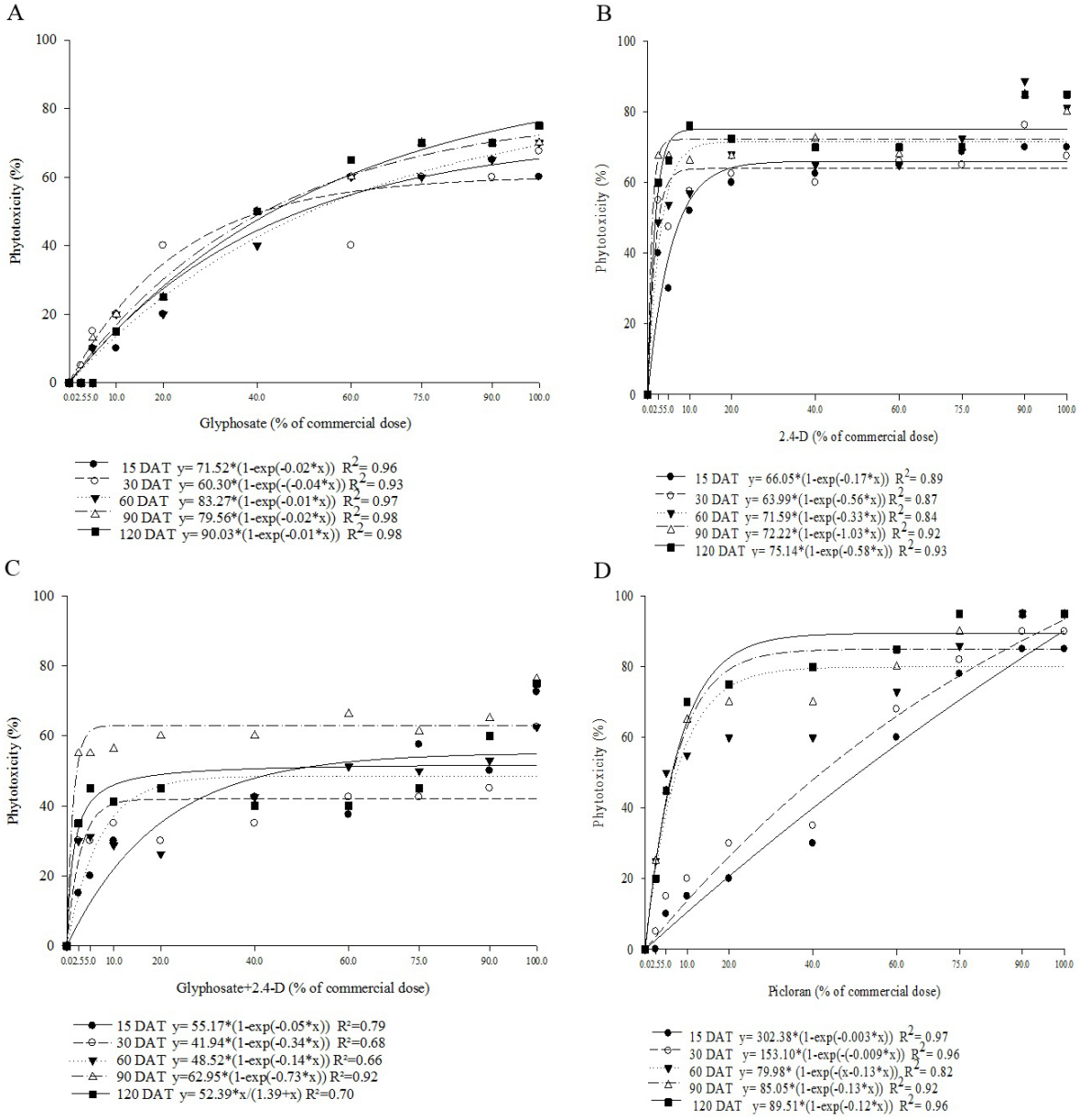

Figure 3. Percentage of phytotoxicity of different doses of glyphosate (A), 2.4-D (B), glyphosate + 2.4-D (C), and picloram (D) on *Myrsine coriacea* evaluated at 15; 30; 60; 90 and 120 days after transplantation (DAT).

Figura 3. Porcentagem de fitotoxicidade de diferentes doses de glyphosate (A), 2,4D (B), glyphosate+2,4-D (C) e picloran (D) à *Myrsine coriacea*, avaliada aos 15; 30; 60; 90 e 120 dias após o transplante (DAT).

Table 3. Shoot biomass, leaf area, height, chlorophyll content and stem diameter of *M. coriaceae* under different doses of the herbicides glyphosate, glyphosate + 2.4-D, 2.4-D and picloram.

Tabela 3. Biomassa seca da parte aérea, área foliar, altura, índice de clorofila e diâmetro do caule de *M. coriaceae* sob diferentes doses dos herbicidas glyphosate, glyphosate+2,4D, 2,4-D, glufosinato de amônio e picloran.

% of the commercial dose	Shoot biomass (g)	Leaf area (cm^2)	Height (cm)			Chlorophyll content		Stem diameter (cm)
Glyphosate			**30 DAT**	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	8.91 a	360.05 a	49.00 a	50.50 a	55.00 a	36.25 a	37.00 a	0.55 a
2.5	5.22 a	167.06 a	51.50 a	52.25 a	54.50 a	34.00 a	35.25 a	0.60 a
5	5.49 a	215. 14 a	52.25 a	52.50 a	53.00 a	35.25 a	27.50 a	0.58 a
10	4.69 a	207.04 a	55.25 a	56.75 a	57.75 a	38.75 a	34.75 a	0.60 a
20	4.65 a	129.99 a	49.75 a	50.75 a	52.00 a	25.00 a	25.50 a	0.50 a
40	5.80 a	156.67 a	52.00 a	52.75 a	54.67 a	19.75 a	17.00 a	0.58 a
60	5.93 a	359.24 a	47.50 a	50.75 a	52.25 a	26.25 a	33.75 a	0.53 a
75	5.12 a	165.33 a	47.50 a	49.00 a	50.75 a	29.25 a	32.00 a	0.58 a
90	6.87 a	149.58 a	51.50 a	52.00 a	51.25 a	30.25 a	33.25 a	0.75 a
100	4.91 a	224.00 a	44.50 a	46.00 a	46.25 b	27.50 a	27.75 a	0.70 a
CV (%)	48.69	58.77	17.58	17.32	16.45	48.49	48.66	25.12
Glyphosate + 2,4D	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	4.74 a	211.35 a	50.00 a	53.50 a	60.75 a	23.50 a	33.50 a	0.60 a
2.5	4.97 a	148.59 a	56.25 a	54.50 a	59.33 a	23.75 a	33.00 a	0.63 a
5	7.39 a	198.29 a	58.75 a	58.50 a	62.25 a	27.75 a	37.25 a	0.73 a
10	6.38 a	212.35 a	57.25 a	55.75 a	59.00 a	33.50 a	31.00 a	0.63 a
20	7.50 a	218.09 a	56.75 a	55.50 a	60.50 a	36.25 a	39.25 a	0.65 a
40	5.51 a	193.31 a	48.00 a	49.50 a	51.00 b	25.50 a	29.25 a	0.55 ab
60	6.56 a	189.60 a	50.50 a	50.50 a	51.75 ab	27.50 a	20.50 a	0.70 a
75	3.98 ab	197.46 a	47.50 a	48.50 a	52.75 ab	36.50 a	27.50 a	0.53 ab
90	4.31 ab	118.26 ab	50.50 a	49.25 a	53.50 ab	25.75 a	31.50 a	0.63 a
100	2.91 b	68.02 b	47.25 a	49.00 a	51.00 b	21.00 a	16.00 a	0.45 b
CV (%)	41.52	59.98	12.68	15.23	15.55	54.80	52.98	17.72
2,4-D	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	5.85 a	199.97 a	52.25 a	53.00 a	55.50 a	36.50 a	34.50 a	0.65 a
2.5	5.64 a	126.78 a	44.25 a	50.00 a	53.25 a	23.50 a	28.25 a	0.50 a
5	4.95 a	196.31 a	45.25 a	45.50 a	49.00 a	27.25 a	32.75 a	0.50 a
10	4.90 a	118.95 a	45.25 a	45.25 a	47.00 a	27.25 a	29.50 a	0.50 a
20	4.46 a	120.70 a	49.25 a	48.50 a	50.75 a	31.75 a	27.25 a	0.55 a
40	2.51 a	133.90 a	45.00 a	46.00 a	48.75 a	18.75 a	19.50 a	0.53 a
60	4.97 a	198.31 a	45.25 a	43.75 a	47.75 a	26.00 a	33.75 a	0.50 a
75	5.32 a	189.07 a	47.75 a	45.25 a	51.25 a	21.00 a	23.50 a	0.58 a
90	3.72 a	110.33 a	43.00 a	39.25 a	46.67 a	25.75 a	24.50 a	0.55 a
100	4.83 a	125.33 a	45.13 a	44.25 a	44.00 b	24.25 a	26.25 a	0.55 a
CV (%)	53.18	65.14	19.24	19.09	19.13	73.13	59.95	18.47
Picloram	**Shoot biomass (g)**	**Leaf area (cm^2)**	**Height (cm)**			**Chlorophyll content**		**Stem diameter (cm)**
			30 DAT	**60 DAT**	**120 DAT**	**15 DAT**	**120 DAT**	
0	8.28 a	432.50 a	45.25	53.75 a	68.25 a	39.25 a	32.75 a	0.48 a
2.5	8.48 a	430.50 a	51.00 a	62.75 a	62.25 a	29.50 a	31.00 a	0.48 a
5	6.03 a	342.25 a	44.38 a	55.25 a	63.75 a	37.75 a	28.25 a	0.35 ab
10	8.10 a	339.50 a	44.50 a	59.75 a	65.50 a	47.50 a	42.00 a	0.35 ab
20	6.50 a	327.75 ab	43.75 a	52.50 a	72.75 a	42.00 a	35.75 a	0.30 ab
40	7.10 a	288.25 ab	47.25 a	54.50 a	63.75 a	37.00 a	40.00 a	0.38 ab
60	6.73 a	225.75 ab	49.25 a	54.75 a	61.00 a	45.00 a	44.00 a	0.35 ab
75	1.95 b	98.50 bc	45.75 a	51.50 a	56.00 ab	31.50 a	37.00 a	0.35 ab
90	1.74 b	79.25 cd	41.25 a	47.75 a	40.25 ab	35.50 a	12.50 b	0.35 ab
100	1.88 b	73.00 d	45.63 a	50.50 a	37.50 b	36.00 a	6.50 b	0.15 b
CV (%)	34.68	62.51	17.38	15.79	23.73	30.58	37.74	33.67

Means with the same letter in the column do not differ according to Tukey's test at a 5% level of significance.

virgilioides and *Plathymenia reticulata* that were subjected to the application of up to 1,440 g ha^{-1} of glyphosate, although *Solanum lycocarpum* showed a difference in these variables with herbicide application.

Regarding the aerial part dry biomass and leaf area, the herbicides did not show a strong effect in general; the most pronounced effect for the herbicide picloram was observed at 75% of the commercial dose, with a decrease of biomass of 77.3% at the highest dose used. An effect on the chlorophyll content was only observed for the herbicide picloram.

4 Conclusions

Among the evaluated herbicides picloram was the most phytotoxic for all species, whereas glyphosate showed lower phytotoxicity at smaller doses for *M. coriacea* relative to *C. floribundus* and *H. americanus*. A negative impact was observed with 2.4-D application in all species even at the smaller doses. The mixture glyphosate + 2.4-D also presented high phytotoxicity at the lowest values for the species *M. coriacea*.

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