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Differential tolerance of four tree species to glyphosate and mesotrione used in agrosilvopastoral systems

Karine Meira de Abreu[1] · Darliane de Castro Santos[1] · João Paulo Pennacchi[2] · Francine Neves Calil[3] · Thamires Marques Moura[4] · Estenio Moreira Alves[4] · Thales Caetano de Oliveira[5] · Fabiano Guimarães Silva[5] · Jaqueline Balbina Gomes Ferreira[1] · Arlini Rodrigues Fialho[1] · Stéfany Oliveira de Souza[1]

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Abstract
Agrosilvopastoral systems present high potential as a sustainable agriculture technique, despite the current limiting points in the systems management that must be overcome. A major bottleneck in the implementation of these systems is the damage caused, to forest species, by the application of herbicides for weed control in the crop component. The present study evaluated the selectivity of herbicides in seedlings of two tree species native to the Brazilian Cerrado, *Dipteryx alata* (Vog.) and *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul, and two eucalyptus clones, *E. urograndis* and *E. urocam*. The study was conducted in a greenhouse, with two commonly-used herbicides (glyphosate and mesotrione), in five different doses. The sensitivity of tree species to the herbicides was evaluated through physiological, morphological and visual phyto intoxication characteristics. For glyphosate, the tree species exhibited strong phytotoxicity symptoms from the second day after application, being the symptoms more prominent in the two eucalyptus clones and less in *D. alata*, which presented higher tolerance to this herbicide. For mesotrione, the four tree species studied showed mild toxicity symptoms, with very little or absent significant impacts in growth and biomass accumulation. The herbicide mesotrione was shown to be selective for the four tree species studied and can be recommended to agroforestry systems in which these species are present. These results based on phytotoxicity grades are reinforced by the biometric and physiological parameters measured.

Keywords *Dipteryx alata* · *Anadenanthera colubrina* · *Eucalyptus* · Glyphosate · Mesotrione

✉ Darliane de Castro Santos
darliane.castro@ifgoiano.edu.br

Extended author information available on the last page of the article

Introduction

Agriculture has been undergoing major transformations motivated by the increase in production costs and a more competitive market. This requires an improvement in the agricultural sector's productivity, quality and profitability, without compromising the environment (Balbino et al. 2012). An efficient productive alternative is the use of agrosilvopastoral systems, which combine trees, pasture and cattle in the same area, constituting a biodiverse system.

Well conducted agrosilvopastoral systems can present a series of advantages such as: optimized use of resources, intensified land use, increased animal welfare, complementarity and synergy between biotic components and rehabilitation of degraded agricultural production areas. This can greatly improve the economic return to rural producers, constituting a competitive differential for Brazilian agribusiness (Calil et al. 2016; Deiss et al. 2018). However, studies about agrosilvopastoral systems are necessary, mainly to find more appropriate crop management techniques for the different species involved, contributing to the further expansion of these production systems.

One of the areas with the greatest need for research development is related to the use of chemical products for the management of agricultural crops in agrosilvopastoral systems, since the drift of applied pesticides can be harmful to the arboreal component of the system. This scenario is worrisome due to the low selectivity of herbicides to forest crops, which can result in injuries at the early development of trees, potentially reducing their productivity (Agostinetto et al. 2010; Minogue et al. 2018).

In Brazil, corn and soybeans are the most common crops used in agrosilvopastoral systems, mainly due to their importance to Brazilian agriculture and adaptation to the integrated systems (Balbino et al. 2011). Mesotrione and glyphosate are the most commonly used products for the control of broad-leaf and grasses weeds, in corn and soybeans, respectively (Beaudegnies et al. 2009; Pignati et al. 2017).

With regard to forest species, eucalyptus is the most used in integrated systems due to its high yield, adaptability to different environments, advances in the area of genetics and its great diversity of species, making it possible to meet the technological requirements of the most different segments of the industrial production (Silva 2018). Among the Brazilian forest species with potential for success in these systems, we can highlight: *Dipteryx alata* (Vog.), which present high quality and resistant wood, in addition to fruits with high nutritional value, for both human and animal consumption (Sano et al. 2004); and *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul whose wood is suitable for civil construction, production of cellulose and firewood, and the possibility of using its bark for medicinal purposes (Dias et al. 2012).

In this context, the objective of this study was to evaluate the selectivity of the herbicides glyphosate and mesotrione, in different doses, for native tree species of the Brazilian Cerrado biome (*D. alata* and *A. colubrina*) and exotic tree species clones (*Eucalyptus urograndis* and *Eucalyptus urocam*), elucidating the potential use of these species in agrosilvopastoral systems with soybean or corn.

Material and methods

Plant material and growing conditions

The study was conducted in a greenhouse at the Plant Tissue Culture Laboratory at the Federal Institute of Education, Science and Technology Goiano (IF Goiano), Campus Rio

Verde (17° 47′S and 50° 54′W), in the State of Goiás, Brazil. Four tree species were used in the experiment: Baru (*Dipteryx alata*), Angico (*Anadenanthera colubrina*) and two eucalyptus clones, *E. urograndis* (I144—*Eucalyptus urophylla* S.T.Blake x *E. grandis* W.Hill ex Maiden) and *E. urocam* (VM01—*E. urophylla* x *E. camaldulensis* Dehnh).

Vegetative material was identified by the authors by comparisons with specimens in the herbaria of the IF Goiano Campus Rio Verde. The taxonomy for family and genus followed APG IV—Angiosperm Phylogeny Group (2016) and plant names were updated according to the Brazilian Flora database (Flora do Brasil 2020, under construction). Seedlings of native species came from a single matrix of each species, produced via seeds and kept in a nursery until reaching 3 months of growth. The eucalyptus clones were purchased from a nursery and were 3 months old.

Plants were transplanted into 8-L polyethylene pots containing latosol as substrate (Table 1), which was corrected with 10 g lime per pot (equivalent to 2,500 kg ha^{-1}), and fertilization at planting was done with 2 g NPK 04–30-10 fertilizer per pot (equivalent to 20, 150 and 50 kg ha^{-1} Nitrogen—N, Phosphorus—P and Potassium—K, respectively). At 56 and 66 days after transplantation, fertilization top dressing was performed with 2 g per pot (equivalent to 500 kg ha^{-1}) of fritted trace-elements (FTE, chemical fertilizer based on micronutrients Boron—B, Manganese—Mn, Zinc—Zn and Sulfur—S), ensuring nutritional quality to the seedlings, minimizing possible any visual effects arising from nutritional deficiency.

The herbicides used were: glyphosate (N-(fosfonometil)glicina), a post-emergence, classified as non-selective and systemic action, with a broad spectrum of action, and control of annual or perennial weeds, both broad and narrow leaves, commonly used in soybean management (Galli and Montezuma 2005); and mesotrione (2-(4-mesyl-2-nitrobenzoyl) cyclohexane-1,3-dione), classified as a selective herbicide, with application in post-emergence, for the control of annual broad leaves and grasses in the corn crop (MAPA - Ministério da Agricultura, Pecuária e Abastecimento 2004; Silveira et al. 2012).

For the glyphosate (commercial product Zapp® QI Soluble Concentrate—SL) the doses used were of 0, 480, 960, 1440, 1920 and 2400 g acid equivalent (a.e.) ha^{-1} and for mesotrione (commercial product Callisto® Concentrate Suspension—SC) the doses were of 0, 48, 96, 144, 192 and 240 g a.e. ha^{-1}. The decision on the doses was made following the commercial application dose recommended by the manufacturers (intermediate doses), and two smaller and two larger doses than recommended, in addition to the control treatment which had no herbicides applied. The use of the sub and super dosages of both products aimed to simulate herbicide drift (sub doses) and cumulative applications, defining safety intervals for the use of both products. These doses were converted, in volume, for each pot. Glyphosate was selected to test a system where tree seedlings are planted with soybean, while mesotrione was chosen to simulate intercrop with corn.

The herbicides were applied 28 days after transplanting the seedlings, when they had an average height of 0.40 m for two eucalyptus clones, 0.55 m for *A. colubrina* and 0.25 m

Table 1 Chemical and physical characteristics of the soil before the onset of the experiment

pH $CaCl_2$	OM ($g\ dm^{-3}$)	P ($m\ dm^{-3}$)	K ($cmol_c\ dm^{-3}$)	Ca	Mg	Al	H+Al	SB	CEC	BS %	Silt	Clay	Sand
5.28	16.8	0.73	0.01	1.06	0.2	0.01	1.34	1.27	2.61	48.7	12	65	23

OM = Organic Matter; BS = base saturation; SB = Sum of bases; CEC = Cation Exchange Capacity for *D. alata*. At the time of application, the climatic conditions were: average wind speed 1 km h^{-1}, air temperature 25.7 °C and relative humidity 78.9%. The application was carried out with a CO_2 pressurized sprayer equipped with a 110–02 (JDF) Ad 02-d simple fan air induction nozzle, regulated to a constant pressure of 2.5 bar and a flow of 200 L ha^{-1}.

After application, the plants remained protected from contact with rainwater or from irrigation in a greenhouse for 24 h, in order to avoid the washing of the product. During the experimental period, the average temperature inside the greenhouse was 28.6 °C and the relative humidity was 58%.

Morphological and biomass assessments

Destructive morphological assessments were performed 80 days after application (DAA) of the herbicide. Shoot height and root length were measured with a ruler. Shoot height was measured from the collar of the seedlings to the apex of the last fully expanded leaf. Root length was measured at the height of the collar of the seedlings to the tip of the longest root. The fresh material was stored in paper bags and dried in a forced air oven at 65 °C, to constant mass, to measure the dry matter of shoot and roots.

Visual diagnosis of phytotoxicity

Visual evaluations of the phytotoxicity effects in the seedlings was performed on 2, 4, 8, 16, 32 and 64 DAA, considering the control sample as a reference, with grades based on the plant intoxication rating scale of the European Research Weed Council (EWRC 1964), which varies from 1 to 9, where: 1—no symptoms; 2—very mild toxicity symptoms; 3—mild toxicity symptoms; 4—moderate toxicity symptoms; 5—doubtful toxicity symptoms; 6—symptoms that appear to indicate strong toxicity; 7—strong toxicity; 8—very strong toxicity; 9—death of plants.

Physiological assessments

The analysis of pigments were performed at 1, 2, 7, 14 and 21 DAA. Chlorophyll levels were determined using a portable chlorophyll meter ClorofiLOG1030® (Falker®, Porto Alegre, RS, Brazil), obtaining the content of chlorophyll *a* and chlorophyll *b*, expressed as the Falker chlorophyll index (FCI). The total chlorophyll and flavonoid indices (flavonols and anthocyanins) were determined using the Dualex Scientific™ sensor (Force-A, Orsay, France), based on the chlorophyll fluorescence emission spectra. The nitrogen balance index (NBI) was calculated as the ratio of chlorophyll to flavonoid levels (Cerovic et al. 2012).

At the same dates mentioned before, the chlorophyll *a* fluorescence transient OJIP was also determined using a portable fluorometer FluorPen FP 100 (Photon Systems Instruments; Drasov, Czech Republic). The OJIP transient is a tool for analyzing the change in kinetics of chlorophyll *a* fluorescence that provides detailed information on the structure and function of the photosynthetic apparatus, especially photosystem II (PSII) (Lazár 2006).

This analysis was performed in young, fully expanded and non-detached leaves, previously adapted to dark for 30 min, for complete oxidation of the photosynthetic electron transport system. Minimum fluorescence (F_o) was measured at 50 µs when all PSII reaction centers are open and is defined as step O, followed by step J (at 2 ms), step I (at 30 ms) and maximum fluorescence (F_m) when all PSII reaction centers are closed, known as step P. These values were used to calculate various bioenergetic indices of photosystem II, according to Strasser et al. (2000).

The parameter V_j is related to the fluorescence at the J step (F_j) of the OJIP curve in relation to the maximum fluorescence (F_m) and is calculated as $(F_j-F_o)/(F_m-F_o)$. According to the JIP test equation, we exposed the leaves to a pulse of light saturation (3000 µmol·m^{-2} s^{-1}) with a wavelength of 450 nm for one second after adaptation to obtain the responses related to the transient of chlorophyll *a* fluorescence and quantum yield (Sup. Table 1).

Experimental design and statistical analysis

The design was completely randomized in a $4\times2\times5+1$ factorial arrangement, with four tree species, two types of herbicides, five doses and one control treatment, totalizing 44 treatments with six replicates each, where each seedling constituted an experimental unit. The herbicides were applied directly to the seedlings, aiming to find a limit dose in which the plants showed tolerance. The experiment was carried out until 80 DAA.

For data analysis, software R, version 3.6.1 was used. Data was organized inside each species and herbicide and the effect of the doses was compared. Initially, data normality was tested using the Shapiro–Wilk test in the package "*ggpubr*". For the normal data, the analysis of variance was performed through ANOVA and the *post-hoc* tests through the Tukey method, using the respective packages "stats" and "*agricolae*". For the non-normal data, a few approaches/packages were tested, with the following presenting the most consistent results: the analysis of variance was performed using the Kruskal–Wallis test in the "*ggpub*" package, with the comparison of means made by Dunn test, using the "*dunn.test*" package.

To test the impact of the herbicides and their toxicity to the plants to physiological and growth parameters, Spearman correlation was calculated between pairs of parameters, using the "*Hmisc*" package.

The level of significance considered for the normality test, the analysis of variance, mean tests and the correlation matrix was 0.05.

Results

Shoot and root dry matter

The application of glyphosate had a negative impact on the growth of all studied species. The plants sprayed with glyphosate showed a decrease in shoot dry matter at the end of the experiment (80 DAA) when compared to the control treatment (Fig. 1). The exception was the dose of 480 g a.e. ha^{-1} glyphosate, which caused no difference in shoot dry matter for *D. alata*. The decrease in shoot dry matter of *A. colubrina* in the treatments with glyphosate, when compared to the shoot dry matter of the control plants, was 86, 87 and 97% at the doses of 1440, 1920 and 2400 g a.e. ha^{-1}, respectively (Fig. 1A). For *D. alata*, there was a reduction in shoot dry matter of 69, 85, 80 and 87% at the doses 960, 1440, 1920 and 2400 g a.e. ha^{-1}, respectively (Fig. 1B). For the *E. urograndis* clone, the lowest reduction in shoot dry matter was on the order of 74% in relation to the control, in dose 480 g a.e.

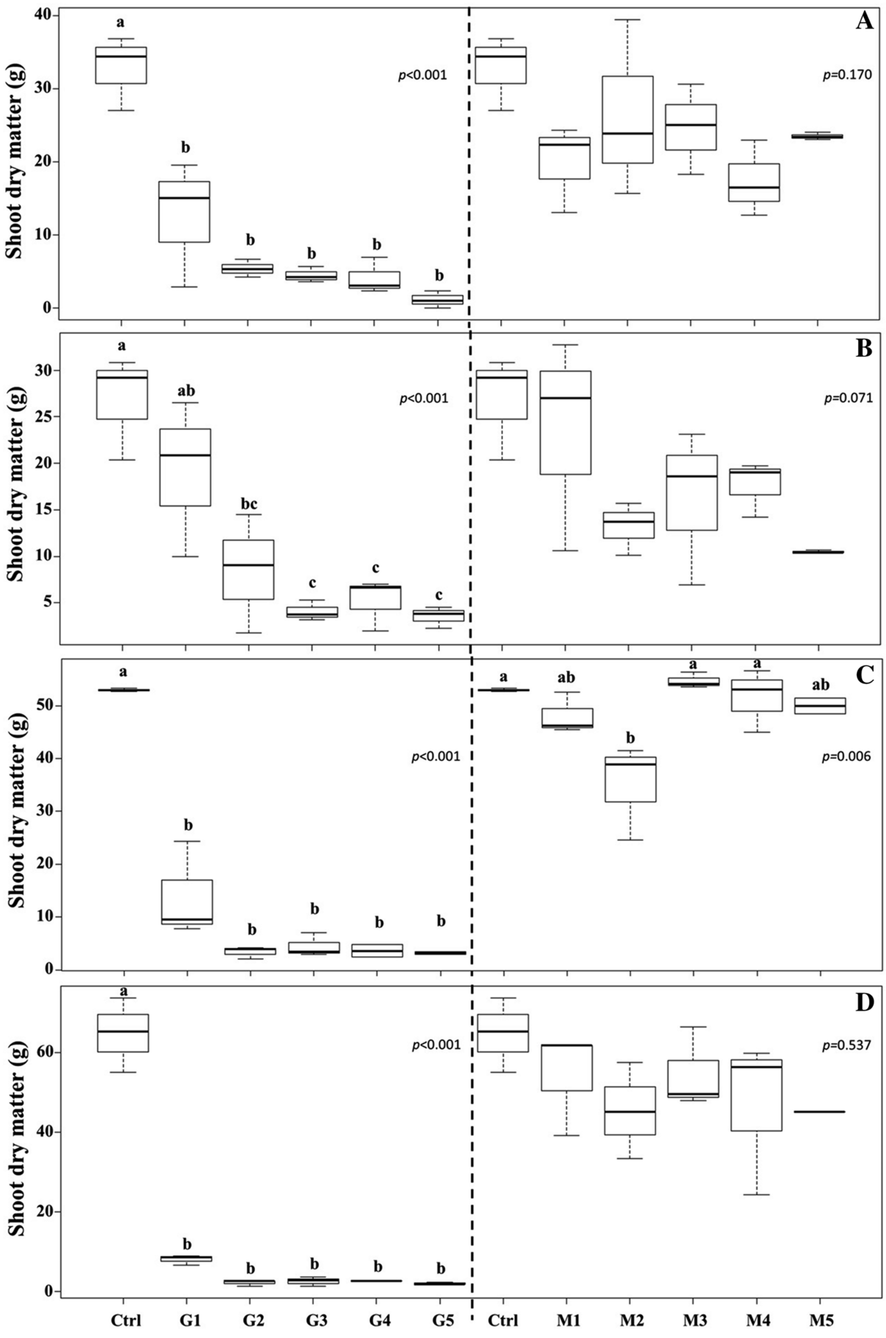

Fig. 1 Boxplot of the shoot dry matter (g) of tree species: **A**—*A. colubrina*; **B**—*D. alata*; **C**—*E. urograndis*; **D**—*E. urocam*, in the treatments with the herbicides glyphosate and mesotrione (G1 to G5-glyphosate at the doses 480, 960, 1440, 1920, 2400 g a.e. ha^{-1}, respectively, and M1 to M5-mesotrione at the doses 48, 96, 144, 192, 240 g a.e. ha^{-1}, respectively). Letters should be used for comparison inside each species and herbicide combination. Treatments with different letters are significantly different from each other for the Tukey test at the *p* level represented in the graphs

ha^{-1}, and for the other doses, it presented around 93% reduction (Fig. 1C). The *E. urocam* clone showed losses of around 96% at doses 1440, 1920 and 2400 g a.e. ha^{-1}, respectively (Fig. 1D).

The herbicide mesotrione proved to be selective for the four studied species. There was no difference for shoot dry matter between the different doses of mesotrione and the control (Fig. 1), with exception of a small decrease for *E. urograndis* clones at 96 g a.e. ha^{-1}.

The application of glyphosate had a negative impact on the root dry matter of the tested materials, but at different levels. For *A. colubrina* the root dry matter was not affected with the application of glyphosate in the dose 480 g a.e. ha^{-1} (Fig. 2A) while for *D. alata* there was no reduction in root dry matter (Fig. 2B). For *E. urograndis* and *E. urocam* there was a decrease in root dry matter for all doses of glyphosate applied when compared to the control (Fig. 2C–D, respectively).

The root dry matter did not differ from control in treatments with mesotrione application (Fig. 2A–D).

Shoot height and root length

For shoot height there was no difference in all treatments with mesotrione and glyphosate for *A. colubrina* and *D. alata* (Fig. 3A–B). Glyphosate had a significant effect ($p < 0.05$) only for *E. urocam* with reductions in the order of 54 to 64% along the doses (Fig. 3D). The herbicide mesotrione only had effect on shoot height for the *E. urograndis*, which showed an increase in shoot height in the treatment with 192 g a.e. ha^{-1} compared to control plants (Fig. 3C).

Regarding the root length, there was a negative effect of glyphosate for *A. colubrina*, *E. urograndis and E. urocam* (Fig. 4A, C, D). Considering *E. urograndis*, there was an decrease of 93, 87, 87 and 97% in relation to the control, at doses 960, 1440, 1920 and 2400 g a.e. ha^{-1}, respectively, while the clone *E. urocam* showed decreases of around 96% compared to the control treatment at these same doses. For *D. alata,* there was no difference in root length of the plants sprayed with glyphosate.

The application of mesotrione caused no differences for root growth for all tree species studied when compared to the control treatment (Fig. 4).

Phytotoxicity

In general, the four tree species evaluated showed strong phytotoxicity symptoms when sprayed with glyphosate and few symptoms with mesotrione. The effect of glyphosate on *A. colubrina* was manifested as: leathery leaves, deformed apices, well-developed necrosis on the edges of the leaves and marked leaf senescence starting at 8 DAA (Table 2). *A. colubrina* seedlings showed 40% mortality, mainly in doses 1440, 1920 and 2400 g a.e. ha^{-1}, the latter causing mortality of all plants throughout the experiment. The seedlings of *A. colubrina* sprayed with mesotrione showed mild symptoms of phytotoxicity with white spots on the leaves at 4 DAA (Table 2). Despite these symptoms, this herbicide did not affect the growth of the species, which recovered until the end of the 64 DAA.

The spraying of mesotrione on *D. alata* caused mild symptoms of phytotoxicity with white spots on the leaves at the highest doses, but there was no change in seedling growth. Although *D. alata* seedlings sprayed with glyphosate showed strong toxicity symptoms (Table 2), there was no plant mortality and the seedlings recovered throughout the experiment, showing regrowth at 64 DAA.

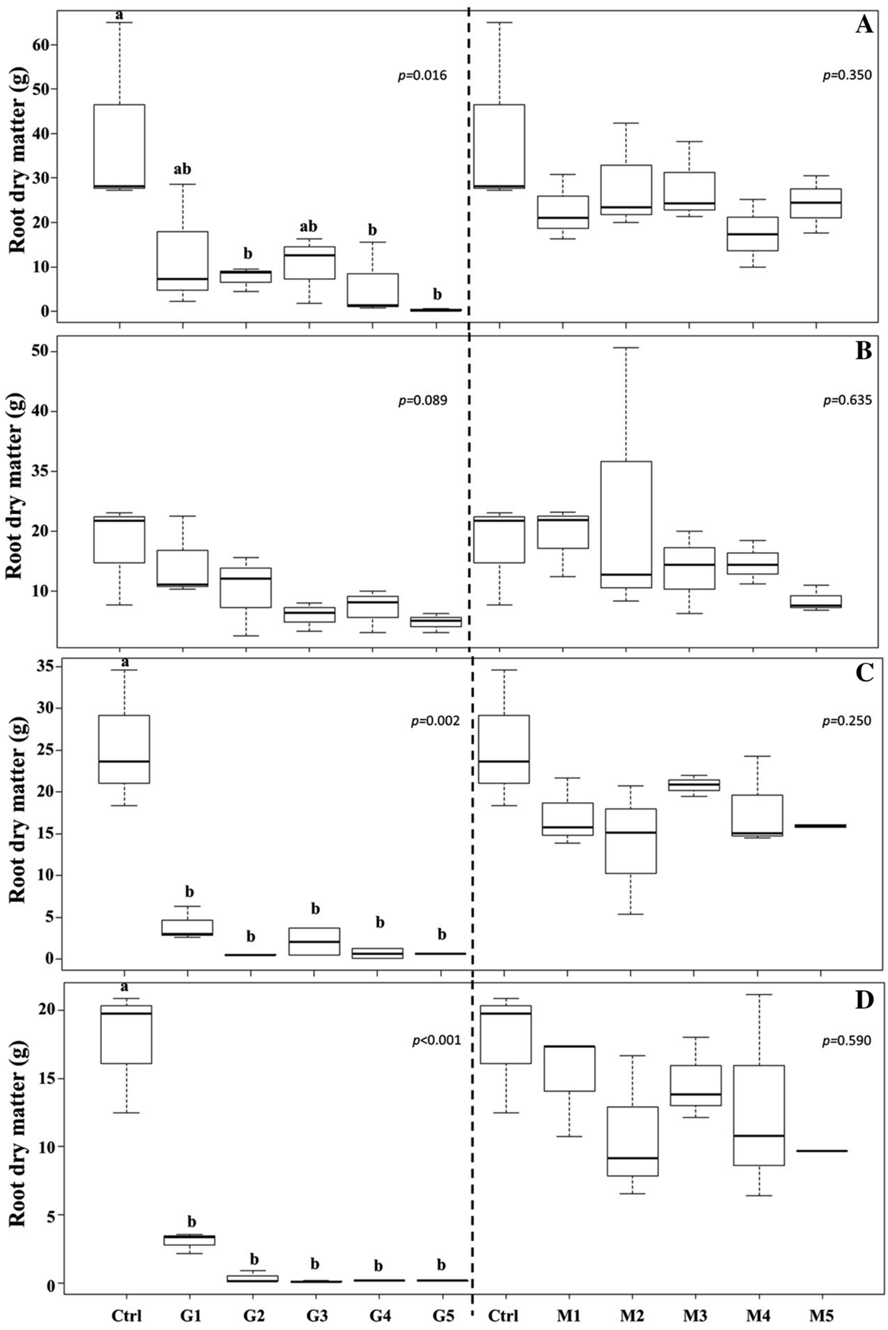

Fig. 2 Boxplot of the root dry matter (g) of tree species: **A**—*A. colubrina*; **B**—*D. alata*; **C**—*E. urograndis*; **D**—*E. urocam*, in the treatments with the herbicides glyphosate and mesotrione (G1 to G5-glyphosate at the doses 480, 960, 1440, 1920, 2400 g a.e. ha^{-1}, respectively, and M1 to M5-mesotrione at the doses 48, 96, 144, 192, 240 g a.e. ha^{-1}, respectively). Letters should be used for comparison inside each species and herbicide combination. Treatments with different letters are significantly different from each other for the Tukey test at the *p* level represented in the graphs

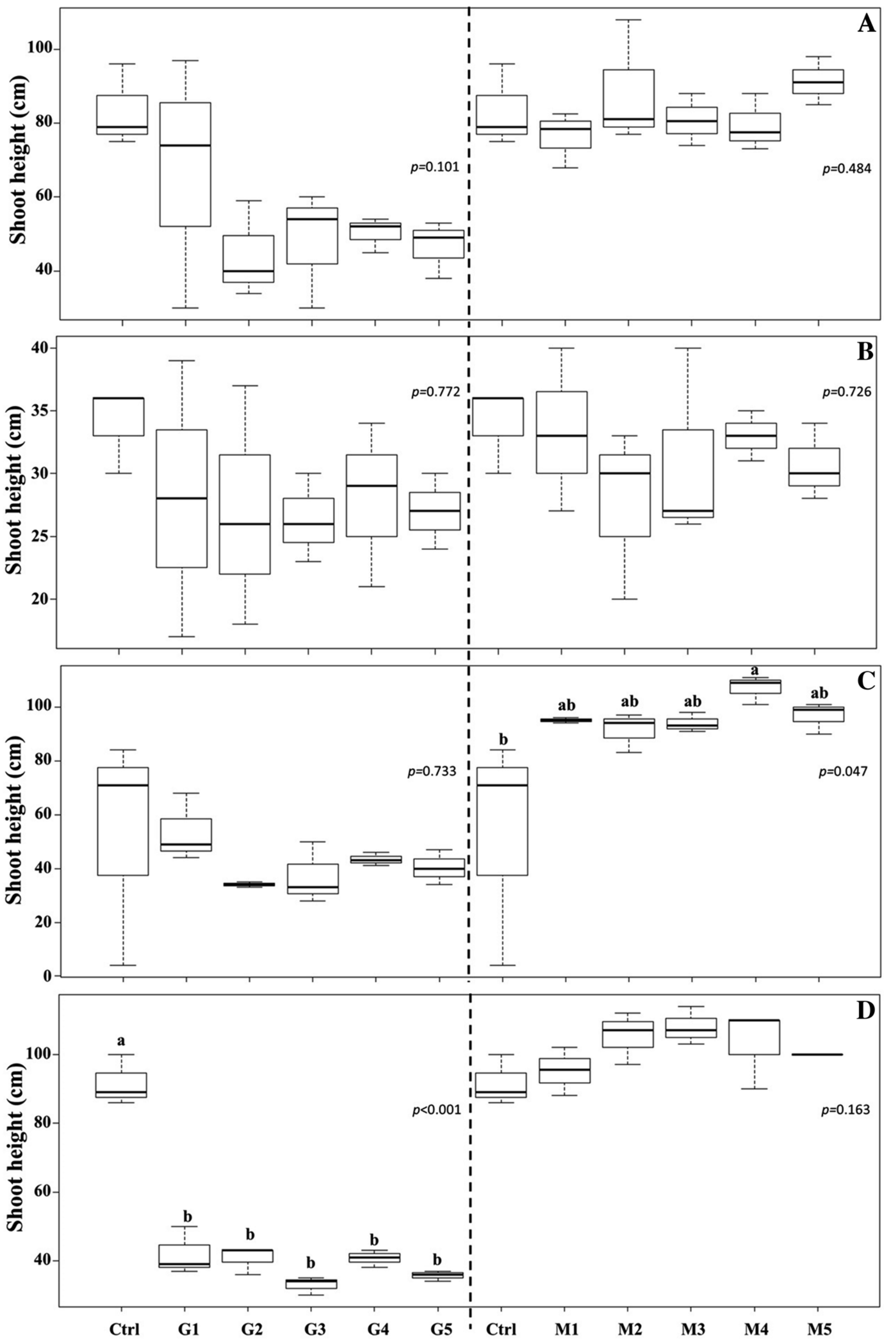

Fig. 3 Boxplot of the shoot height (cm) of tree species: **A**—*A. colubrina*; **B**—*D. alata*; **C**—*E. urograndis*; **D**—*E. urocam*, in the treatments with the herbicides glyphosate and mesotrione (G1 to G5-glyphosate at the doses 480, 960, 1440, 1920, 2400 g a.e. ha^{-1}, respectively, and M1 to M5-mesotrione at the doses 48, 96, 144, 192, 240 g a.e. ha^{-1}, respectively). Letters should be used for comparison inside each species and herbicide combination. Treatments with different letters are significantly different from each other for the Tukey test at the *p* level represented in the graphs

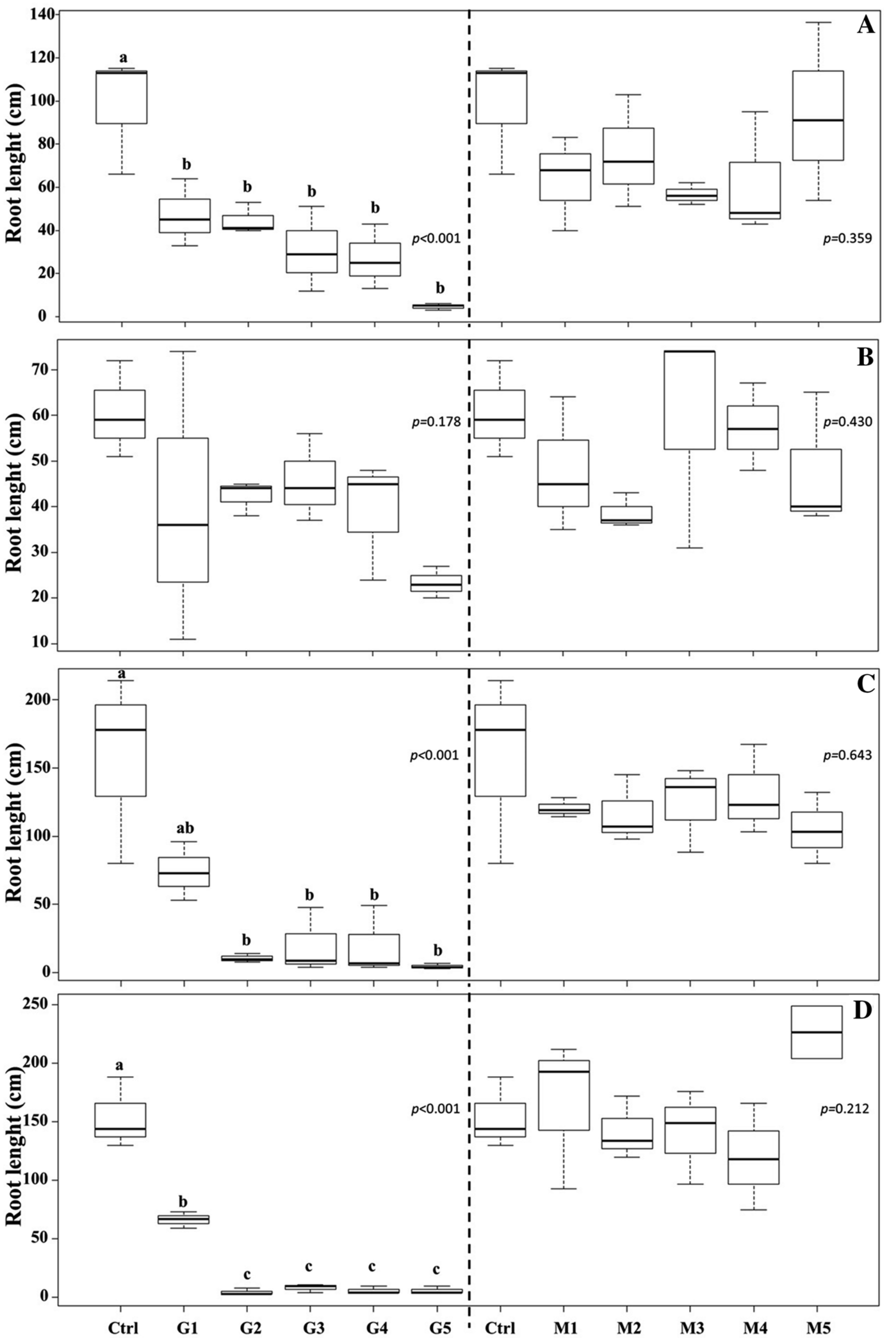

Fig. 4 Boxplot of the root lenght (cm) of tree species: **A**—*A. colubrina*; **B**—*D. alata*; **C**—*E. urograndis*; **D**—*E. urocam*, in the treatments with the herbicides glyphosate and mesotrione (G1 to G5-glyphosate at the doses 480, 960, 1440, 1920, 2400 g a.e. ha^{-1}, respectively, and M1 to M5-mesotrione at the doses 48, 96, 144, 192, 240 g a.e. ha^{-1}, respectively). Letters should be used for comparison inside each species and herbicide combination. Treatments with different letters are significantly different from each other for the Tukey test at the *p* level represented in the graphs

Table 2 Average scores of the phytotoxicity levels of the plants of *A. colubrina*, *D. alata* and the *Eucalyptus* clones (urograndis and urocam) sprayed with glyphosate and mesotrione depending on the dose and the time of evaluation

Species	DAA	Glyphosate (g a.e. ha^{-1})						Mesotrione (g a.e. ha^{-1})					
		0	480	960	1440	1920	2400	0	48	96	144	192	240
A. colubrina	2	1.0	1.0	1.0	1.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	4	1.0	3.0	3.0	1.7	3.0	3.0	1.0	1.0	2.3	3.0	1.7	1.3
	8	1.0	4.7	4.7	4.7	8.0	9.0	1.0	1.0	3.7	1.0	2.3	1.7
	16	1.0	4.7	5.3	5.3	8.7	9.0	1.0	4.0	4.0	3.7	5.3	3.7
	32	1.0	5.3	4.7	7.7	8.7	9.0	1.0	4.0	3.7	3.0	1.7	3.7
	64	1.0	3.0	3.0	4.3	7.7	9.0	1.0	3.0	1.3	1.7	3.0	1.7
D. alata	2	1.0	1.0	1.0	1.3	1.3	1.3	1.0	1.0	1.0	1.0	1.0	1.0
	4	1.0	4.0	3.0	4.0	4.0	4.0	1.0	3.0	1.0	1.0	1.0	3.0
	8	1.0	3.0	3.3	4.7	5.3	5.3	1.0	1.3	1.0	1.7	1.7	1.7
	16	1.0	5.0	8.0	8.0	4.7	8.0	1.0	3.0	1.3	2.3	2.3	2.3
	32	1.0	5.3	5.3	5.3	8.0	8.0	1.0	3.0	1.0	3.7	4.0	3.7
	64	1.0	3.0	3.7	4.3	4.3	8.0	1.0	3.0	1.0	2.3	3.7	3.7
E. urograndis	2	1.0	3.0	3.0	3.0	1.7	3.0	1.0	1.0	1.0	1.0	1.0	1.0
	4	1.0	3.0	8.0	3.0	3.0	3.0	1.0	1.0	5.0	1.0	3.0	3.0
	8	1.0	5.3	8.0	8.0	8.0	9.0	1.0	3.0	3.0	3.0	3.0	3.0
	16	1.0	5.3	8.7	8.7	8.7	9.0	1.0	3.0	4.0	3.0	2.3	2.3
	32	1.0	5.0	9.0	8.7	8.7	9.0	1.0	3.0	2.3	3.0	3.0	3.0
	64	1.0	1.7	9.0	9.0	5.3	9.0	1.0	1.0	1.0	1.0	1.0	1.0
E. urocam	2	1.0	1.7	1.3	1.7	1.3	3.0	1.0	1.0	1.0	1.0	1.0	1.0
	4	1.0	8.0	3.0	8.0	8.0	8.0	1.0	4.0	1.0	1.0	4.0	3.0
	8	1.0	5.3	8.0	8.3	9.0	9.0	1.0	1.7	2.0	1.7	3.0	3.0
	16	1.0	8.0	9.0	9.0	9.0	9.0	1.0	1.0	1.0	3.0	2.7	2.3
	32	1.0	8.0	9.0	9.0	9.0	9.0	1.0	3.0	1.7	1.3	1.7	3.0
	64	1.0	3.0	9.0	9.0	9.0	9.0	1.0	3.0	1.7	1.0	1.7	2.3

Scores of the phytotoxicity: 1—no symptoms; 2—very mild toxicity symptoms; 3—mild toxicity symptoms; 4—moderate toxicity symptoms; 5—doubtful toxicity symptoms; 6—symptoms that appear to indicate strong toxicity; 7—strong toxicity; 8—very strong toxicity; 9—plant death (EWRC 1964)

For two eucalyptus clones, treatments with mesotrione caused only mild symptoms in plants at 8 DAA (Table 2), which did not affect the growth of these clones. However, the herbicide glyphosate for *E. urograndis* was quite harmful, regardless of the concentration evaluated, with the mortality percentage reaching 67% and with the symptoms of phytotoxicity starting at 2 DAA, with dryness of the leaves evolving into leathery leaves, deformed apices, well developed necrosis on the edges of the leaves and marked leaf senescence.

E. urocam also showed strong phytotoxicity symptoms in all treatments after glyphosate application (Table 2), with phytotoxicity symptoms found from 2 DAA, with the twisting of tender tissues, branches and new leaves, followed by the death of the branches, with a total mortality of 80% of the seedlings. Only in the plants that received the lowest dose (480 g a.e. ha^{-1}) it was possible to observe recuperation during the experiment, and at 64 DAA they had new shoots.

A correlation analysis between the scores and the biomass allocation parameters allowed to evaluate the effect of herbicide toxicity on seedling growth (Table 3). For glyphosate, in general, there was a high negative correlation between toxicity and the shoot and root dry matter. This means that the higher the toxicity score, the lower the shoot and root dry matter. In *E. urograndis* seedlings, this correlation was already observed at 2 DAA, for *A. colubrina* and *E. urocam*, from the 4 DAA, and for *D. alata* from the 8 DAA. The correlation values indicate the level of influence of toxicity on the growth of the seedlings, the most affected being the eucalyptus clones, followed by *A. colubrina* and lastly *D. alata* (Table 3). For mesotrione, the correlations presented much lower values, or were even non-significant as in the case of *D. alata*, demonstrating a reduced impact of the herbicide on seedling growth (Table 3).

Physiological assessments

Leaf pigments

There were correlations between plant dry matter and pigments quantification, with a negative trend for Eucalyptus, and less intense for native species. Therefore, the reduction in pigments combined with death or reduction in plant growth provides negative correlations. On the other hand, the trend of positive correlations in natives may express the

Table 3 Spearman correlation between scores of phytotoxicity of *A. colubrina*, *D. alata* and the *Eucalyptus* clones (urograndis and urocam) and the biomass allocation parameters in the different times of evaluation, in the treatments with glyphosate and mesotrione.

			Days after application (DAA)					
			2	4	8	16	32	64
A. colubrina	Glyphosate	SDM	–	**– 0.73**	**– 0.82**	**– 0.80**	**– 0.85**	**– 0.64**
		RDM	–	**– 0.60**	**– 0.78**	**– 0.77**	**– 0.72**	**– 0.61**
	Mesotrione	SDM	–	–	–	**– 0.49**	–	**– 0.51**
		RDM	–	–	–	–	–	–
D. alata	Glyphosate	SDM	–	–	**– 0.69**	**– 0.65**	**– 0.74**	**– 0.47**
		RDM	–	–	**– 0.55**	**– 0.54**	**– 0.66**	–
	Mesotrione	SDM	–	–	–	–	–	–
		RDM	–	–	–	–	–	–
E. urograndis	Glyphosate	SDM	**– 0.79**	**– 0.54**	**– 0.92**	**– 0.93**	**– 0.90**	**– 0.78**
		RDM	**– 0.76**	**– 0.56**	**– 0.89**	**– 0.89**	**– 0.84**	**– 0.58**
	Mesotrione	SDM	–	**– 0.66**	–	–	–	–
		RDM	–	**– 0.50**	**– 0.53**	–	–	–
E. urocam	Glyphosate	SDM	–	**– 0.73**	**– 0.93**	**– 0.98**	**– 0.98**	**– 0.78**
		RDM	–	**– 0.71**	**– 0.92**	**– 0.97**	**– 0.97**	**– 0.81**
	Mesotrione	SDM	–	–	–	–	–	**– 0.62**
		RDM	–	–	–	–	–	**– 0.65**

DAA Days after application. *SDM* Shoot dry matter (g); *RDM* Root dry matter (g). All correlations in the Table are significant at the 5% probability level. The—symbols represent non significant correlations maintenance of the pigment apparatus. These results are supported by observations related to chlorophyll *a* fluorescence and quantum yield (Sup. Table 2). Correlations between phytotoxicity grades and pigment parameters are not conclusive (Sup. Table 3, 4, 5 and 6).

For the scores, some correlations were more recurrent and will be described below. For pigment analyses, the application of glyphosate in *A. colubrina* caused high toxicity in the seedlings and there was a negative correlation between the flavonoid content at 2 DAA and the phytotoxicity scores at 4, 8, 16 and 32 DAA (r = – 0.68, r = – 0.58, r = – 0.55 and r = – 0.63, respectively) (Sup. Table 3). There was also a negative correlation between anthocyanin content at 14 DAA and toxicity at 32 DAA (r = – 0.65) (Sup. Table3). For the herbicide mesotrione in the species *A. colubrina*, there was also a negative correlation between toxicity at 16 DAA and flavonoid content at 2 DAA (r = – 0.59) (Sup. Table 3).

In *D. alata* subjected to glyphosate application, there was a correlation between toxicity at 32 DAA and the flavonoid content at 7 DAA (r = – 0.48) (Sup. Table 4). There was a positive correlation between flavonoids at 14 DAA and toxicity at 8, 16 and 32 DAA (r = 0.56, r = 0.49 and r = 0.48, respectively) and also the flavonoid content at 21 DAA and toxicity at 8, 32 and 64 DAA (r = 0.60, r = 0.61 and r = 0.51, respectively) (Sup. Table 4). A negative correlation pattern was found for NBI at 14 DAA and toxicity at 16, 32 and 64 DAA (r = – 0.47, r = – 0.59 and r = – 0.48, respectively) (Sup. Table 4). With respect to mesotrione in *D. alata*, there was a negative correlation between flavonoids at 14 DAA and toxicity at 8, 32 and 64 DAA (r = – 0.53, r = – 0.50 and r = – 0.64, respectively) (Sup. Table 4).

For *E. urograndis*, in treatments with glyphosate there was a positive correlation between flavonoids at 7 DAA and toxicity at 2, 8, 16 and 32 DAA (r = 0.60, r = 0.62, r = 0.59 and r = 0.60, respectively) and also flavonoids at 14 DAA and toxicity at 2, 8, 16, 32 and 64 DAA (r = 0.72, r = 0.81, r = 0.81, r = 0.83 and r = 0.67, respectively) (Sup. Table 5). For treatments with mesotrione, there was a negative correlation between flavonoids at 7 DAA and toxicity at 4 DAA (r = – 0.47) and between anthocyanins at 14 DAA and toxicity at 8 DAA (r = – 0.49) (Sup. Table 5).

In *E. urocam*, in glyphosate treatments, there were positive correlations between NBI at 1 DAA and toxicity at 4, 8, 16, 32 and 64 DAA (r = 0.60, r = 0.71, r = 0.77, r = 0.77 and r = 0.54, respectively) (Sup. Table 6). In addition, there was a negative correlation between flavonoid content at 2 DAA and toxicity at 4 DAA (r = – 0.53), and positive correlations between flavonoid content at 14 and 21 DAA and toxicity at 2 DAA (r = 0.82 and r = 0.91, respectively) (Sup. Table 6). For mesotrione, there was a positive correlation between NBI at 1 DAA and toxicity at 8 DAA (r = 0.73) and between NBI at 2 DAA and toxicity at 8 and 64 DAA (r = 0.53 and r = 0.59, respectively). There was also a negative correlation between flavonoids at 7 DAA and toxicity at 8 DAA (r = – 0.52) (Sup. Table 6).

The significant correlations between PI_{ABS} and the toxicity grades for mesotrione were obtained in the moment of maximum expression of toxicity symptoms (8 e 16 DAA). These correlations were positive for *A. colubrina*, *D. alata* and *E. urograndis* (Sup. Table 7, 8 and 9). The only negative correlation (– 0,73) between PI_{ABS} (2 DAA) and toxicity grade (16 DAA) was obtained for *E. urocam* (Sup. Table 10). No significant correlations between PI_{ABS} and toxicity were observed for glyphosate application, in any studied species (Sup. Table 7, 8, 9 and 10). The FV/FO ratio presented a similar behaviour in terms of the correlations to toxicity, as PI_{ABS}.

Clones of *E. urograndis* and *E. urocam*, when exposed to different doses of glyphosate, show isolated negative correlations between phytotoxicity scores (2, 8, 16, 32 and 64 DAA) and as primary fluorescence assessments, mainly 1 DAA (Sup. Table 9 and 10). In this way, it is possible to correlate the reduction in this parameter with the increase in the intensity of phytotoxicity notes. *A. colubrina* and *D. alata* had no effect on this correlation (Sup. Table 7 and 8). Thus, V_J is an important variable for detecting the sensitivity and potential for phytotoxicity of the studied species.

There was only one pattern of negative correlation between the parameter V_j and the phytotoxicity levels for eucalyptus species. For the treatment with glyphosate in *E. urograndis*, there was a negative correlation between V_j at 1 DAA and toxicity at 2, 8, 16 and 32 DAA (r = − 0.54, r = − 0.77, r = − 0.77 and r = − 0.70, respectively) and even higher between V_j at 7 DAA and toxicity at 2, 8, 16 and 32 DAA (r = − 0.85, r = − 0.86, r = − 0.86 and r = − 0.76) (Sup. Table 9). For mesotrione, in this same specie, there was a negative correlation between V_j at 1 DAA and toxicity at 8 and 32 DAA (r = − 0.66 and r = − 0.57, respectively) and V_j at 7 DAA and toxicity at 8 and 16 DAA (r = − 0.59 and r = − 0.53, respectively) (Sup. Table 9).

For *E. urocam*, the effect of glyphosate resulted in a negative correlation between V_j at 1 DAA and toxicity at 8, 16, 32 and 64 DAA (r = − 0.57, r = − 0.54, r = − 0.54 and r = − 0.72, respectively) and for mesotrione between V_j at 1 DAA and 8 DAA (r = − 0.53) (Sup. Table 10).

This research demonstrated the potential of using the herbicide mesotrione as an alternative for weed management in agrosilvopastoral systems containing these four tree species, considering that, in general, the morphological growth of the tree species *A. colubrina*, *D. alata*, *E. urograndis* and *E. urocam* were not harmed by the use of this herbicide.

The application of glyphosate had a greater harmful effect on the development of seedlings, where *E. urocam* proved to be more sensitive among the studied species, with all morphological variables negatively affected by this herbicide. The native species *D. alata* showed an indicative of tolerance to glyphosate, followed by *A. colubrina*.

Discussion

In this study, the sensitivity of four tree species to glyphosate and mesotrione were tested, simulating the impact of chemical weed control management on tree growth in agrosilvopastoral systems. The discovery of the physiological behavior of chlorophyll *a* fluorescence will facilitate the understanding of the tolerance level of these species. In this way, it sheds light on the use of these molecules (Glyphosate and Mesotrione) in the management of spontaneous plants in silvicultural and/or agroforestry systems that have them present.

From the point of view of the influence of glyphosate on the growth and accumulation of biomass in the studied species, there was a response to inhibit the growth of seedlings of both native and exotic species, and the main response was the abscission of leaflets and the delay in growth in height of the plants, as also observed by Tuffi Santos et al. (2006) and Costa et al. (2012) working with glyphosate drift simulation in eucalyptus. Glyphosate has primary effects on plants, inhibiting or slowing their growth, this delay being due to the inhibition of the synthesis of amino acids responsible for the synthesis of proteins essential for plant growth (Velini et al. 2009; Vivancos et al. 2011), mainly related to the shikimic acid cycle (Gomes et al. 2014).

In the present study, the correlations between morphological variables (shoot and root dry matter at 80 DAA) to phytotoxicity scores, showed a negative relationship, that is, the higher the herbicide toxicity to the plant, the lower its increment, due the capacity of biomass accumulation was affected by decreases in carbon fixation capacity by damages to the leaves tissues and leaf loss, consequently, decreasing plant height and length of the root system.

Tuffi Santos et al. (2006) analyzed five different eucalyptus clones, and observed that glyphosate, at doses above 86.4 g ha^{-1}, caused severe plant intoxication, affecting their growth, resulting in lower height, stem diameter and dry matter at 45 days after herbicide application, where the greater the sensitivity of the genetic material to the herbicide, the lower the growth of the plants, and the lower the accumulation of dry matter. Yamashita et al. (2009) observed that, at 28 DAA of a glyphosate dose of 360 g ha^{-1}, *Ceiba pentandra* and *Schizolobium amazonicum* plants presented reduction in height and chlorosis in the leaves.

In the present study, symptoms in the plants of the glyphosate treatments evolved to dehydrated leaves, deformed apices, well-developed necrosis at the edges of the leaves and marked leaf senescence, and as a consequence there was a decrease in the shoot and root dry matter. Leaf symptoms like these were also observed in eucalyptus plants subjected to simulated drift with glyphosate reported by Tuffi Santos et al. (2006), Tuffi Santos et al. (2007), Costa et al. (2012) and Inoue et al. (2014). In a study with native species, similar symptoms as observed in the present study were verified for Marchi et al. (2018) after glyphosate application in *A. colubrina* and by Duarte et al. (2006) in *Anadenanthera macrocarpa* (Benth.) Brenan. Machado et al. (2013) observed that *Solanum lycocarpum* showed high phytotoxicity with the herbicide glyphosate, even at the lowest doses of 160 and 480 g ha^{-1}.

For *D. alata*, although phytotoxicity symptoms of glyphosate were observed, this did not alter its growth on the same scale as for other species, being the only species with survival of all seedlings in all treatments, already presenting signs of recovery and regrowth at 64 DAA, leading to the hypothesis of potential tolerance of *D. alata* to glyphosate under the specific conditions of this study. There are similar reports in the literature for resistance to glyphosate application in *Kielmeyera lathrophyton* Saddi (Machado et al. 2013) and *Jatropha curcas* L. (Agostinetto et al. 2010).

Between the eucalyptus clones, the *E. urocam* showed higher sensitivity to glyphosate with a 80% of mortality against 66% in *E. urograndis*. For Tuffi Santos et al. (2006), the difference in sensitivity between eucalyptus genotypes, through the application of glyphosate, can be attributed to differences in uptake, translocation, compartmentalization, root exudation and metabolism of the herbicide molecule.

In turn, mesotrione was selective to the four species studied, with no alteration and neither delay in species development at 80 DAA. This herbicide acts by inhibiting the biosynthesis of carotenoids through the interference with the activity of the enzyme HPPD (4—hydroxyphenylpyruvate-dioxygenase) in chloroplasts (Karam and Cruz 2004). Although it acts on the photosystems, no harmful effects of the herbicide were observed on the species. Such results of mesotrione demonstrate its lower phytotoxicity and allow its recommendation for use in agrosilvopastoral systems with the studied species trees, since it manages to effectively control the competing vegetation in corn cultivation, and does not harm the development of trees under the same conditions of the studied environment.

As verified on the physiological traits analysis, an increase in toxicity by the herbicide resulted in a decrease in NBI. This may indicate an increase in secondary metabolism, to the detriment of the primary metabolism, as a defense of the plant, since stress in plants can act by redirecting the photosynthetically fixed carbon from the synthesis of primary metabolites, such as cellulose, lipids and proteins, toward the synthesis of secondary metabolites such as flavonoids and other phenolic compounds (Pacheco et al. 2011). This may be explained by the lower impact suffered by *D. alata* in glyphosate treatments.

Proto (2012) also noted that *D. alata* plants were less sensitive to glyphosate, showing stability of physiological characteristics even with visual leaf damage caused by the herbicide. The lower intensity of the effects in *D. alata* is probably related to the greater thickness of the epicuticular wax layer in this species (Varanda and Santos 1996), representing a barrier in the process of herbicide absorption. However, studies are still required to elucidate the average tolerance of *D. alata* to such high doses of glyphosate, which proved to be harmful to other species.

Although our results do not show clear trends of correlation with significant decrease in chlorophyll, the chlorosis presented by the plants under study with glyphosate may be the result of chloroplast degeneration or inhibition of chlorophyll formation (Cole et al. 2000).

The negative correlations in eucalyptus clones between fluorescence at 2 ms (stage J) and toxicity show a negative effect of glyphosate on electron transport, mainly related to PSII as also observed by Bethke et al. (2013) and Christensen et al. (2003). The results indicate that the greater the toxic effects of the herbicides, the lower the relationship between the maximum fluorescence of chlorophyll *a* and the fluorescence of chlorophyll *a* at point J of the induction curve. This phase of the curve is related to the electron transfer between quinones *a* and *b* in the PSII, and the negative correlation presented represents a loss of quantum efficiency in the transfer of electrons in this phase of the chain.

The accumulation of electrons not transported by the chain increases the formation of singlet oxygen causing the photochemical extinction that is initiated due to the increase in electrons exported from the PSII (Konrad et al. 2005). The use of parameters related to chlorophyll *a* fluorescence, especially OJIP tests, are generally recommended for the evaluation of plants under different types of stresses (Strasser et al. 2000) and specifically for the herbicides present in this study such as glyphosate (Kargar et al. 2019) and mesotrione (Silvestre et al. 2014). However, the extreme symptoms generated, mainly by glyphosate application, with posterior death of leaves and plants, may have blurred the correlation among phytotoxicity and fluorescence parameters.

The reduction in the photosynthesis performance index (PI_{ABS}) can be a result of the destruction of PSII reaction centres, motivated by application of herbicides with formulations based in mesotrione (Hassannejad et al. 2020). This indicates that *E. urocam* clone be the most susceptible species to mesotrione in the present study.

This research has focused on the study of a broad band of doses of the herbicides aiming to establish a limit tolerance for the species. This strategy has shown reasonable results when comparing phytotoxicity to plant growth and biomass allocation. However, for further studies of physiological interactions the use of narrow bands of herbicide doses, closer to the limit of tolerance, may yield better results by avoiding plant death and extreme damage to leaves and photosynthetic machinery.

Conclusion

It is concluded that glyphosate provides greater damage/death as well as changes in the physiological parameters evaluated when compared to mesotrione. Glyphosate tolerance was limited to *D. alata*. The species *A. colubrina*, *D. alata*, *E. urograndis* and *E. urocam* are tolerant to different doses of mesotrione. The *E. urocam* clone is the most sensitive to the molecules studied.

Based on the mentioned results, field studies must be performed to determine the limit age/size of the tree species for which they can possibly be affected by phytotoxic effects of the herbicides commonly used for corn and soybean in agrosilvopastoral systems. The impact of different spraying techniques, that could reduce the effect of herbicides in tree species, may also be a matter of future investigation, generating further knowledge and new strategies for this agricultural activity.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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Authors and Affiliations

Karine Meira de Abreu[1] · Darliane de Castro Santos[1] · João Paulo Pennacchi[2] · Francine Neves Calil[3] · Thamires Marques Moura[4] · Estenio Moreira Alves[4] · Thales Caetano de Oliveira[5] · Fabiano Guimarães Silva[5] · Jaqueline Balbina Gomes Ferreira[1] · Arlini Rodrigues Fialho[1] · Stéfany Oliveira de Souza[1]

Karine Meira de Abreu
karinemeiradeabreu@gmail.com

João Paulo Pennacchi
jppennacchi@gmail.com

Francine Neves Calil
fncalil@gmail.com

Thamires Marques Moura
thamires.moura@ifgoiano.edu.br

Estenio Moreira Alves
estenio.moreira@ifgoiano.edu.br

Thales Caetano de Oliveira
thalescaetano100@gmail.com

Fabiano Guimarães Silva
fabiano.silva@ifgoiano.edu.br

Jaqueline Balbina Gomes Ferreira
balbinajaqueline@gmail.com

Arlini Rodrigues Fialho
arlinirodrigues@hotmail.com

Stéfany Oliveira de Souza
stefany.souzza00@gmail.com

[1] Laboratório de Química Agrícola, Instituto Federal de Educação, Ciência e Tecnologia Goiano, Rodovia Sul Goiana, Km 01, Zona Rural, Rio Verde, Goiás CEP: 75.901-970, Brasil

[2] Laboratório de Ecofisiologia, Universidade Federal de Lavras, Caixa Postal 3037, Lavras, Minas Gerais CEP: 37200-900, Brasil

[3] Laboratório de Ecologia de Plantas, Universidade Federal de Goiás, Av. Esperança, s/n - Chácaras de Recreio Samambaia, Goiânia, Goiás CEP: 74690-900, Brasil

[4] Instituto Federal de Educação, Ciência e Tecnologia Goiano, Avenida Oeste, nº 350, Parque União, Iporá, Goiás CEP: 76200-000, Brasil

[5] Laboratório de Cultura de Tecidos Vegetais, Instituto Federal de Educação, Ciência e Tecnologia Goiano, Rodovia Sul Goiana, Km 01, Zona Rural, Rio Verde, Goiás CEP: 75.901-970, Brasil