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Growth, morphological, metabolic and photosynthetic responses of clones of eucalyptus to glyphosate

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ABSTRACT

We hypothesized that eucalyptus has clone-dependent responses to glyphosate, and such differential responses might be associated with morphological, metabolic and/or photosynthetic changes. Experiments were carried out under controlled conditions of temperature, photoperiod and nutrition, focusing on evaluating the response of *Eucalyptus* × *urograndis* clones (GG100 and I144) to increasing doses of glyphosate (0–1440 g ha^{-1} acid equivalent – AE) and to test whether a differential plant response would be associated to alterations in leaf morphology, plant and herbicide metabolism and photosynthesis. There was a significant reduction of plant height, stem diameter, number of leaves, leaf area and shoot dry mass caused by low doses of glyphosate ($\leq$180 g AE ha^{-1}, while a strong plant growth reduction (~60%) was caused by glyphosate field doses ($\geq$720 g AE ha^{-1}), in both clones. The GG100 clone was more susceptible to glyphosate field doses, while the I144 clone was more susceptible to glyphosate low doses. The stomatal index increased by 31% and the nervure thickness was reduced by 17% at 30 days after application of glyphosate at 180 g AE ha^{-1} (DAA) in the GG100 clone. Traces of glyphosate (< 28 g mg^{-1} of dry mass) were found in leaf tissues of both clones at 1 DAA. Shikimic acid accumulated earlier (after 1 DAA) and in greater amounts (90%) in the I144 clone. Aminomethylphosphonic acid (AMPA) was not detected in either treated clone. The CO_2 assimilation rate, transpiration rate and stomatal conductance were reduced earlier (after 1 DAA) and more intensely (65%) in the I144 clone. The clone-dependent response is apparently associated with changes in plant metabolism related to glyphosate mode of action and gas exchange response differences between the clones.

1. Introduction

Commercial plantations of eucalyptus are of great economic importance to tropical and subtropical regions around the world covering roughly a 20,000,000 ha (Silva et al., 2019). In Brazil, Eucalyptus is the most planted forest species, and plantation acreage continues to expand (Bassaco et al., 2018). Although nine *Eucalyptus* spp. and their hybrids are planted on a large scale worldwide (> 90% of eucalyptus plantations) (Potts and Dungey, 2004; Harwood, 2011), only *Eucalyptus grandis*, *Eucalyptus urophylla* and their hybrids have been the most widespread taxa in Brazilian commercial plantations (Assis et al., 2015). In the past years, the use of *Eucalyptus* × *urograndis* has been predominant in Brazilian tropical sites (Simetti et al., 2018) due especially to its fast growth, improved wood basic density, resistance to drought stress and high yield. Currently, Brazilian commercial plant materials of *Eucalyptus* × *urograndis* are generally obtained from vegetative propagation in clonal mini-gardens, that produce commercial named clones.

Eucalyptus is susceptible to weed interference, especially during its initial growth and development periods from planting until about a year (Nambiar and Sands, 1993; Florentine and Fox, 2003; Garau et al., 2009). During this period, weed control is necessary to prevent a growth reduction or even plant death, which might impact on eucalyptus plantation establishment and yield. Weed control has been commonly performed with post-emergence applications of glyphosate [*N*-(phosphonomethyl)-glycine] (Carvalho et al., 2016). Because

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glyphosate is a non-selective, broad-spectrum herbicide, it may cause injury to any plant species that is not tolerant or resistant to glyphosate (Carvalho et al., 2018), such as eucalyptus. Thus, the biggest problem of using glyphosate in eucalyptus plantations is accidental drift (Tuffi Santos et al., 2005; Salgado et al. 2017) which may occur if the herbicide is sprayed under inadequate environmental conditions or/and the application technology is misused (Machado et al., 2010).

Glyphosate is the most important herbicide in history (Duke and Powles, 2008), partly due to its versatility of use in agricultural and forest areas (Carvalho et al., 2015). It is one of the main herbicides used for weed control in eucalyptus plantations (Costa et al., 2012) due to some favorable characteristics (Tuffi Santos et al., 2007a, 2007b) such as the high efficacy and the large spectrum of control, the low toxicity to mammals, birds, and fishes, the fast degradation by microorganisms and the very short soil persistence (Preston and Wakelin, 2008). In plants, glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS, EC 2.5.1.19) of the shikimate pathway (Duke and Powles, 2008). As a consequence, this herbicide inhibits the biosynthesis of aromatic amino acids required by protein synthesis (Franz et al., 1997), affecting plant growth and survival. Secondary effects of glyphosate have been reported on photosynthesis (Cedergreen and Olesen, 2010; Carvalho et al., 2018; Nascentes et al., 2018), leaf morphology (Tuffi Santos et al., 2005, 2009) and secondary metabolism of plants (Velini et al., 2008; Olesen and Cedergreen, 2010; Gomes et al., 2016).

The first notable metabolic alteration due to phytotoxicity of glyphosate is the accumulation of shikimic acid, and it has been used as a marker for identification of susceptible plants (González-Torralva et al., 2010). Less susceptible plants may occur due to sequestration of glyphosate into vacuoles, limiting glyphosate translocation, although enhanced vacuolar sequestration has only been shown to occur in some species with evolved resistance to glyphosate (e.g. Ge et al., 2010, Ge et al., 2012). Single or double codon changes in the gene for EPSPS have evolved in some weed species as a result of glyphosate selection pressure (reviewed by Duke, 2019). The single and double mutations provide low and high levels of resistance, respectively. Herbicide degradation may play a role in some cases of tolerance or resistance to glyphosate (Cruz-Hipólito et al., 2009; Rojano-Delgado et al., 2010; Pan et al., 2019). In plants, glyphosate can be degraded to the very weak phytotoxin aminomethylphosphonic acid (AMPA) and the non-toxic natural metabolite glyoxylate (Duke, 2011).

Although a differential response of clones of eucalyptus to glyphosate was previously reported (Tuffi Santos et al., 2007a, 2007b; Carvalho et al., 2015, 2018), the physiological basis for the differential responses was not determined. The objective of the present study was to further evaluate the response of two clones of eucalyptus to increasing doses of glyphosate and to test whether alterations in leaf morphology, plant metabolism and photosynthesis might explain any differential response between these clones of eucalyptus.

2. Material and methods

2.1. Plant material and growing conditions

Plantlets of two *Eucalyptus* × *urograndis* clones were obtained from vegetative propagation in a commercial clonal mini-garden and hereafter are designed as GG100 and I144. Both clones have commercial relevance in Brazil. Plantlets with 4–5 fully expanded leaves and approximately 30-cm tall were transferred into 3-L pots filled with washed river sand and daily irrigated with 100 mL of 50% concentration of Hoagland and Arnon (1950) nutrient solution. Plants were grown in a growth chamber at 25 ± 2 °C temperature, 14:10 h (light:dark) photoperiod and 400 μmol m^{-2} s^{-1} photosynthetically active radiation delivered by white fluorescent lights. Experiments were carried out separately for each study.

2.2. Herbicide and application conditions

We used a glyphosate isopropylamine salt herbicide (Roundup Original, Monsanto, Brazil) with the concentration of 480 g active ingredient (360 g acid equivalent – AE) per liter. Glyphosate was sprayed directly onto the eucalyptus shoots by using a CO_2 backpack-sprayer equipped with four flat fan nozzles (110:02, TeeJet, Brazil) at 2 bars pressure and 200 L ha^{-1} spray volume. Herbicide application was performed at 50 cm above the top of plants, after which eucalyptus plantlets were kept for a 30-day acclimation period within the growth chamber. Water with no glyphosate was applied to the non-treated control plants.

2.3. Dose-response assays

Glyphosate was applied at doses of 9, 18, 36, 72, 90, 180, 360, 720 and 1440 g AE ha^{-1}), and a non-treated control was used. At 30 days after glyphosate application (DAA), we evaluated plant height, stem diameter, number of leaves, leaf area and shoot dry mass. Plant heights were measured from the soil level to the top of the plant using a meter stick. Stem diameter was measured at 2-cm up from the soil level of the plant using a caliper. The number of leaves was counted directly. Leaf area was estimated using a leaf area meter (LI3000, Licor, USA). Shoot dry mass was weighted using a semi-analytical balance (AD3300, Marte, Brazil) after the plant material was dried in an air convection oven during seven days at 65 °C. Based on the results of dose–response assays, the glyphosate dose used in the further studies (180 g AE ha^{-1}) was chosen.

2.4. Morphology assays

Glyphosate was applied at 180 g AE ha^{-1}, and a non-treated control was used. At 7, 14 and 30 DAA, we determined stomatal index, leaf blade thickness and nervure thickness of treated and non-treated plants, according to methods used by Carvalho et al. (2018). Measurements were made on the first fully extended leaf by the time of herbicide application, so that new leaves expanded after the herbicide application were not considered. Stomatal index was determined in the middle region of the abaxial leaf surface according to the formula of Salisbury (1927): S.I. = [number of stomata/(number of other epidermal cells + number of stomata)]. Leaf blade thickness and nervure thickness were determined in the middle region of leaf blade, based on methods of Johansen (1940) and Krauter (1985), using FAA 50 (50 mL of 37% formaldehyde + 50 mL of 100% acetic acid + 900 mL of 50% ethanol), transverse cuts and digital images.

2.5. Assays for glyphosate, AMPA, and shikimate content

Glyphosate was applied at 180 g AE ha^{-1}, and a non-treated control was used. At 1, 4 and 7 DAA, we determined the content of glyphosate, shikimic acid and AMPA in leaf tissues of treated and non-treated plants using a high-performance liquid chromatography and mass spectrometry system (LC-MS/MS), according to methodology of Gomes et al. (2015). At those times, all leaves were collected from the whole plant, immediately frozen at −80 °C and then maintained at −20 °C until lyophilization. Lyophilized plant material was powdered, and then 100 mg of plant material was weighed for extraction and simultaneous determination of glyphosate, shikimic acid and AMPA in the leaf dry mass (DM) by LC-MS/MS.

2.6. Photosynthesis assays

Glyphosate was applied at 180 g AE ha^{-1}, and a non-treated control was used. At 1, 4 and 7 DAA, we evaluated the relative chlorophyll content through the SPAD index (Amaral et al., 2019; Sakaigaichi et al., 2019; and others), the maximum quantum efficiency of photosystem II

(Fv/Fm) and gas exchange parameters in both treated and non-treated plants. The SPAD index was measured in four leaves of each plant using a chlorophyll meter (ClorofiLOG, Falker, Brazil). The CO_2 assimilation rate (CO_2 flux), transpiration rate (H_2O flux) and stomatal conductance (GS) were measured on the second expanded-leaf from the top using an infra-red gas analyzer (LI6400, Licor, USA) with 900 μmol m^{-2} s^{-1} photosynthetic active radiation and 25 °C leaf temperature.

2.7. Statistical design and analysis

We used a complete randomized design with 10 replicates in all experiments. Dose-response data were submitted to a non-linear regression (Eq. (1)).

$$y = min + (max - min)/(1 + (x/EC50)^{Hillslope}) \quad (1)$$

where: y is the response variable; x is the herbicide dose; min is the bottom of the curve; max is the top of the curve; EC50 is the value for the curve point that is midway between the max and min parameters; and *Hillslope* characterizes the slope of the curve at its midpoint.

Photosynthesis, metabolism and morphology data were analyzed by ANOVA, in a 2 × 3 factorial scheme, where the factor 1 was the glyphosate dose and the factor 2 was time of evaluation. All tests were performed separately for each clone of eucalyptus. For all analyses, we considered the significance of 5% probability of error.

3. Results

3.1. Dose-response assays

Plant height, stem diameter, number of leaves, leaf area and shoot dry mass were significantly reduced by using higher doses of glyphosate, especially ≥ 180 g AE ha^{-1}, for both eucalyptus clones (Figs. 1 and 2). The dose required to reduce plant height, stem diameter, number of leaves, leaf area and shoot dry mass by 50% (EC50) was found at 119.6, 308.6, 91.2, 143.4 and 134.2 g AE ha^{-1} in the GG100 clone, respectively, and at 136.0, 194.4, 102.0, 132.4 and 90.8 g AE ha^{-1} in the I144 clone, respectively (Table 1).

3.2. Leaf morphology assays

Stomatal index showed difference between treated (9.9%) and non-treated (7.6%) plants of the GG100 clone just at 30 DAA, and no difference was found in the I144 clone (Table 2). Leaf blade thickness was found ranging from 242 up to 323 μm in the GG100 clone and from 308 up to 321 μm in the I144 clone (Table 2) with no difference between treated and non-treated plants in each time of evaluation. Nervure thickness showed difference between treated (716 μm) and non-treated (859 μm) plants of the GG100 clone just at 30 DAA, and no difference was found in the I144 clone (Table 2).

3.3. Glyphosate, AMPA, and shikimate content

Traces of glyphosate were found in leaf tissues of both eucalyptus clones treated with 180 g AE ha^{-1} of a glyphosate (Table 3). At 7 DAA, 27.1 μg mg^{-1} DM of glyphosate was found in the GG100 clone and 20.5 μg mg^{-1} DM of glyphosate was found in the I144 clone (Table 3). Shikimic acid significantly increased when glyphosate was applied, except for the GG100 clone at 1 DAA (Table 3). At 7 DAA, the GG100 treated plants had 134.4 μg g^{-1} DM, an increase of 71% compared to untreated plants (78.7 μg g^{-1} DM) (Table 3), while the I144 treated plants had 145.1 μg g^{-1} DM, an increase of 89% compared to untreated plants (76.7 μg g^{-1} DM) (Table 3). AMPA was not detected in leaf tissues of either clone (Table 3).

3.4. Photosynthesis assays

SPAD index was found ranging from 42.3 up to 50.4 in the GG100 clone and from 43.9 up to 51.6 in the I144 clone (Table 4) with no difference between treated and non-treated plants in each time of evaluation. The Fv/Fm was found ranging from 0.81 up to 0.82 in the GG100 clone and from 0.79 up to 0.82 in the I144 clone (Table 4) with no difference between treated and non-treated plants in each time of evaluation. There were differences in CO_2 flux, H_2O flux and GS between treated and non-treated plants at 4 and 7 DAA in the GG100 clone and at 1 DAA, 4 DAA and 7 DAA in the I144 clone (Table 4). At 7 DAA, treated plants of the GG100 clone showed 5.7 μmol m^{-2} s^{-1} CO_2 flux, 2.6 mmol m^{-2} s^{-1} H_2O flux and 98 mmol m^{-2} s^{-1} GS while non-treated plants showed 8.6 μmol m^{-2} s^{-1} CO_2 flux, 4.2 mmol m^{-2} s^{-1} H_2O flux and 252 mmol m^{-2} s^{-1} GS (Table 4); in addition, treated plants of the I144 clone showed 2.8 μmol m^{-2} s^{-1} CO_2 flux, 1.8 mmol m^{-2} s^{-1} H_2O flux and 39 mmol m^{-2} s^{-1} GS while non-treated plants showed 8.3 μmol m^{-2} s^{-1} CO_2 flux, 3.3 mmol m^{-2} s^{-1} H_2O flux and 224 mmol m^{-2} s^{-1} GS (Table 4).

4. Discussion

Understanding the physiological or other plant traits that explain differential sensitivities of different clones of a crop like eucalyptus to glyphosate could be useful in predicting harmful effects of glyphosate drift. The intention of this study was to add further to what is known of this topic.

There was a significant plant growth reduction caused by low doses of glyphosate (≤180 g AE ha^{-1}) in both clones of eucalyptus, regarding plant height, stem diameter, number of leaves, leaf area and shoot dry mass at 30 DAA. In addition, an expected strong plant growth reduction was found when we applied glyphosate field doses (≥720 g AE ha^{-1}) (Figs. 1 and 2). By inhibiting EPSPS and blocking the shikimate pathway, glyphosate inhibits the biosynthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan required for protein synthesis by plants (Franz et al., 1997), which are essential for plant growth and survival (Herrmann, 1995). The reduction in the content of aromatic amino acids triggers various metabolic processes that may lead to plant death, including a failure to produce compounds derived from the shikimate pathway (*e.g.*, plastoquinone and indole-3-acetic acid), a disruption of carbon flow or interference in carbon allocation, and reduced protein synthesis (Fisher et al., 1986; Becerril et al., 1989).

Growth inhibition is the first visible symptom of the phytotoxic effect of glyphosate (Lydon and Duke, 1988; Gruys and Sikorski,1999) which occurs due probably to a fast decrease in the carbon assimilation, since Boudet et al. (1985) reported that 20% of carbon fixed by photosynthesis that goes to biomass production is derived from the shikimate pathway that is responsible for approximately 35% of the dry mass accumulation by plants. The blocking of the shikimate pathway leads a deficiency in important end products such as lignins, alkaloids, flavonoids and plastoquinone, and a decrease in biomass production in a dose-dependent manner (Olesen and Cedergreen, 2010; Gomes et al., 2017). If lethal doses of glyphosate are applied, progressive symptoms such as yellowing, chlorosis and necrosis develop until plant death occurs (Lydon and Duke, 1988; Gruys and Sikorski, 1999). Negative effects of glyphosate have been reported even with non-lethal doses, causing reductions on morphological and physiological traits of eucalyptus plants (Tuffi Santos et al., 2007b; Velini et al., 2008; Nascentes et al., 2018; Carvalho et al., 2018). If young eucalyptus plants are accidentally exposed to field doses of glyphosate, the negative effects of the herbicide become progressive and culminate in plant death (Salgado et al., 2011).

On the other hand, if non-lethal doses of glyphosate are applied to eucalyptus plants, either growth recovery may occur after exposure to the herbicide, or plant growth may be stimulated by low doses of glyphosate in a process named hormesis. Positive effects of low doses of

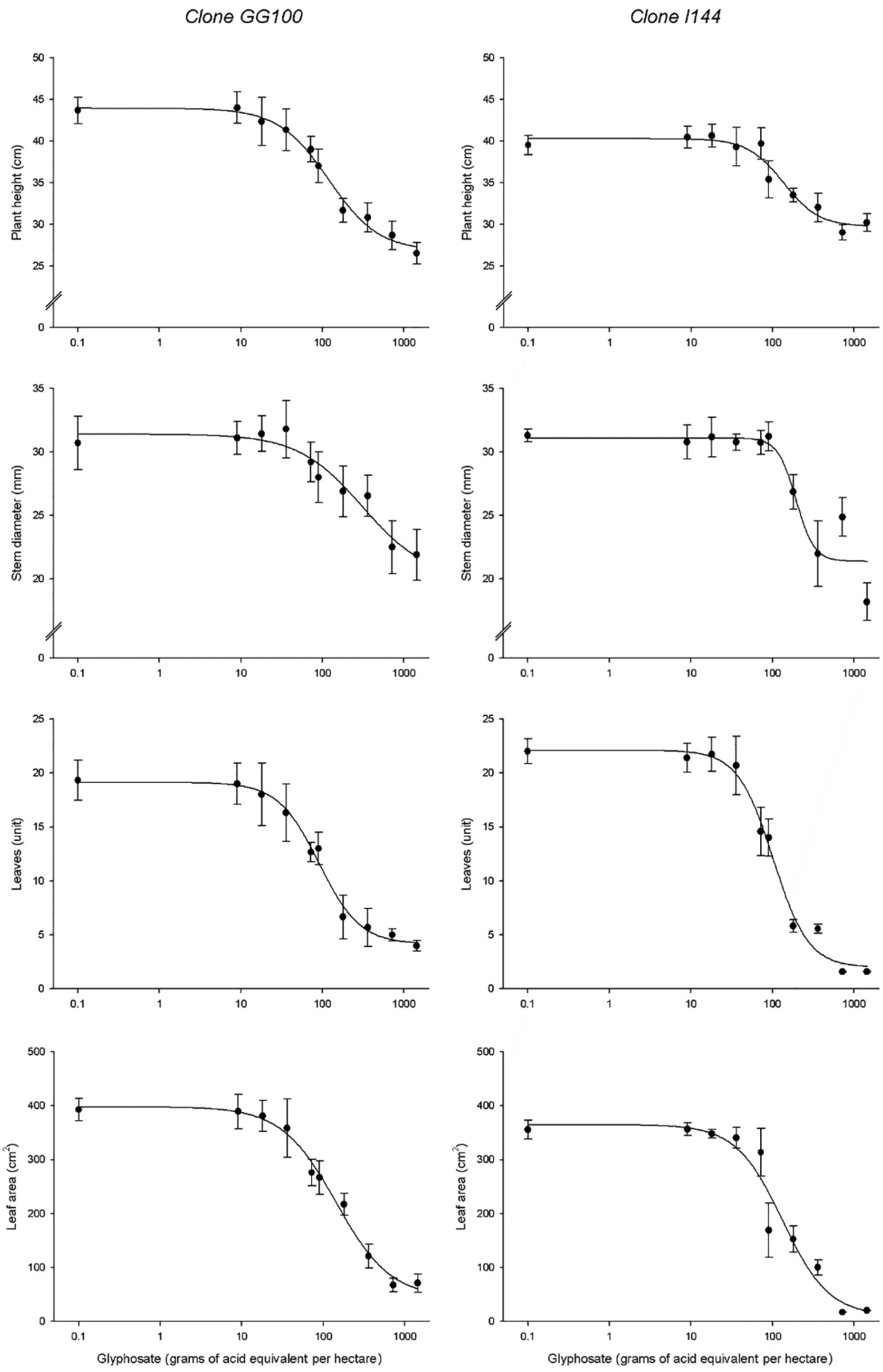

Fig. 1. Plant growth parameters of *Eucalyptus* × *urograndis* clones GG100 and I144 at 30 days after glyphosate application. Error bars indicate standard error of mean ($N = 10$).

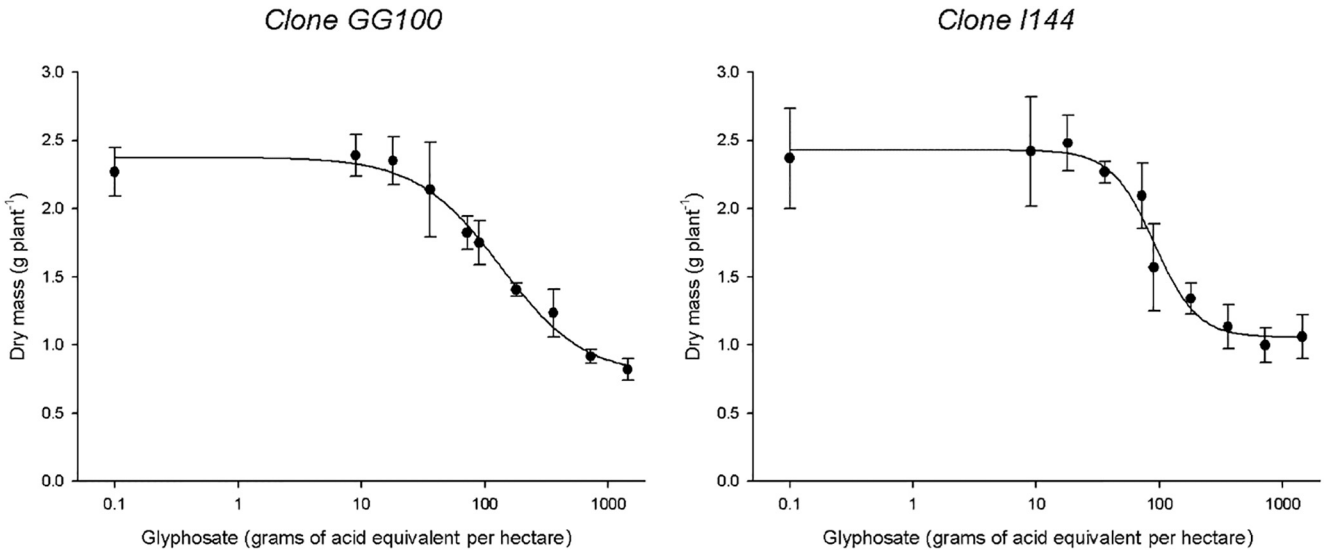

Fig. 2. Shoot dry mass accumulation by plants of *Eucalyptus* × *urograndis* clones GG100 and I144 at 30 days after glyphosate application. Error bars indicate standard error of mean (N = 10).

Table 1
Parameters of the logistic equation [y = min + (max–min)/(1 + abs(x/EC50)^Hillslope)] for the plant growth parameters (PGR) of *Eucalyptus* × *urograndis* clones GG100 and I144 at 30 days after glyphosate application.

PGP	Clones	min	max	EC50[/1]	Hillslope	R^2	*P* value
Plant height	GG100	26.8	43.9	119.6	1.4	0.98	< 0.01
	I144	29.7	40.3	136.0	2.0	0.93	< 0.01
Stem diameter	GG100	20.0	31.4	308.6	1.1	0.92	< 0.01
	I144	21.4	31.1	194.4	3.9	0.83	< 0.01
Leaves	GG100	4.2	19.1	91.2	1.7	0.98	< 0.01
	I144	2.0	22.1	102.0	2.0	0.98	< 0.01
Leaf area	GG100	43.8	397.8	143.4	1.3	0.98	< 0.01
	I144	10.7	364.8	132.4	1.5	0.93	< 0.01
Dry mass	GG100	0.8	2.4	134.2	1.3	0.98	< 0.01
	I144	1.1	2.4	90.8	2.5	0.96	< 0.01

[/1] Concentration of glyphosate (grams of acid equivalent per hectare) required for 50% reduction.

glyphosate on eucalyptus plants has been reported. Doses of glyphosate stimulating plant growth of *Eucalyptus grandis* ranged from 1.8 to 36 g AE ha^{-1}, but the doses at which maximum effects were observed varied considerably for different plants and tissues (Velini et al., 2008). In addition, doses of glyphosate ranging from 6.2 to 20.2 g AE ha^{-1} stimulated plant growth of *Eucalyptus urograndis* clone I144 (Nascentes et al., 2018), a same species genotype we used in this study. However, both the hormetic effect and the magnitude of hormesis are dependent on many factors such as plant species (Velini et al., 2008), species genotypes (Carvalho et al., 2015), time after treatment (Nascentes et al., 2015), age and physiological status of the plants (Carvalho et al., 2013) and environmental factors (Belz and Duke, 2014). Even though hormesis is commonly found with glpphosate, no hormetic effect was observed in this study. This is not surprising, as hormesis is not always observed, making the study of hormesis difficult (Belz and Duke, 2014).

There was a significant plant growth reduction caused by low doses of glyphosate (≤180 g AE ha^{-1}) in both clones of eucalyptus, regarding plant height, stem diameter, number of leaves, leaf area and shoot dry mass at 30 DAA. In addition, an expected strong plant growth reduction was found when we applied glyphosate field doses (≥720 g AE ha^{-1}) (Figs. 1 and 2). The overall estimated plant growth reduction caused by the application of glyphosate at 1,440 g AE ha^{-1} was found to be 62% and 60% for the clones GG100 and I144, respectively, considering the average reduction of all plant growth parameters. Carvalho et al. (2015) found similar tolerance between the clones GG100 and I144 to glyphosate, although they were less tolerant that other *Eucalyptus* × *urograndis* clones (e.g. C219 and I224). However, the magnitude of reduction was found dependent on the parameter evaluated. For example, the reduction was higher in the GG100 clone than the I144 clone regarding on plant height (13%), stem diameter (5%) and shoot dry mass (13%), while a higher plant growth reduction was found in the I144 clone regarding on number of leaves (13%) and leaf area (8%), comparing the maximum and minimum estimated values of each parameter (Table 1). Thus, stem-related parameters reduced more in the GG100 clone while leaf-related parameters were reduced more in the I144 clone due especially to a high defoliation of this clone.

Table 2
Leaf morphology parameters (± standard error of mean, N = 10) of *Eucalyptus* × *urograndis* clones GG100 and I144 after glyphosate application.

Time[/1]	Glyphosate dose[/2] (g AE ha^{-1})	Stomatal Index[/3] (%)		Leaf blade thickness (μm)		Nervure thickness[/3] (μm)	
		GG100	*I144*	*GG100*	*I144*	*GG100*	*I144*
7	0	7.5 ± 0.7	8.1 ± 1.0	294 ± 37	312 ± 36	756 ± 46	812 ± 67
	180	7.9 ± 0.9	8.2 ± 0.8	242 ± 26	308 ± 29	672 ± 63	785 ± 59
14	0	7.7 ± 0.7	8.3 ± 1.1	307 ± 20	321 ± 35	757 ± 62	823 ± 62
	180	8.8 ± 1.0	8.4 ± 1.3	302 ± 31	318 ± 42	691 ± 45	801 ± 72
30	0	7.6 ± 0.8b	7.9 ± 0.9	323 ± 41	318 ± 53	859 ± 53 a	884 ± 46
	180	9.9 ± 0.9 a	8.4 ± 1.2	319 ± 23	316 ± 42	716 ± 57b	816 ± 58

[/1] Time represents the days after glyphosate application.
[/2] AE means acid equivalent of glyphosate.
[/3] Different lowercase letters indicate significant differences between glyphosate doses in each time of evaluation by Tukey test at 5% probability of error.

Table 3
Metabolic parameters (± standard error of mean, $N = 10$) of *Eucalyptus* × *urograndis* clones GG100 and I144 after glyphosate application.

Time[/1]	Glyphosate dose[/2] (g AE ha^{-1})	Glyphosate[/3] (μg mg^{-1} DM)		Shikimic acid[/4] (μg g^{-1} DM)		AMPA[/5]	
		GG100	*I144*	*GG100*	*I144*	*GG100*	*I144*
1	0	ND	ND	69.3 ± 5.6	74.6 ± 6.3b	ND	ND
	180	9.1 ± 3.1	12.0 ± 2.3	80.8 ± 6.2	96.8 ± 7.1 a	ND	ND
4	0	ND	ND	81.2 ± 7.8b	71.6 ± 6.7b	ND	ND
	180	21.6 ± 2.8	20.3 ± 3.5	125.6 ± 10.6 a	141.4 ± 11.3 a	ND	ND
7	0	ND	ND	78.7 ± 6.8b	76.7 ± 7.2b	ND	ND
	180	27.1 ± 2.6	20.5 ± 3.7	134.4 ± 13.5 a	145.1 ± 9.8 a	ND	ND

[/1] Time represents the days after glyphosate application.
[/2] AE means acid equivalent of glyphosate.
[/3,/4,/5] represent the content of glyphosate, shikimic acid and aminomethyl-phosphonic acid (AMPA), respectively, in dry leaf material (DM = dry mass); ND indicates no detected traces; Different lowercase letters indicate significant differences between glyphosate doses in each time of evaluation by Tukey test at 5% probability of error.

Table 4
Photosynthetic parameters (± standard error of mean, $N = 10$) of *Eucalyptus* × *urograndis* clones GG100 and I144 after glyphosate application.

Time[/1]	Glyphosate dose[/2] (g AE ha^{-1})	SPAD index[/3]		Fv/Fm[/4]		CO_2 flux[/5] (μmol m^{-2} s^{-1})		H_2O flux[/6] (mmol m^{-2} s^{-1})		GS[/7] (mmol m^{-2} s^{-1})	
		GG100	*I144*	*GG100*	*I144*	*GG100*	*I144*	*GG100*	*I144*	*GG100*	*I144*
1	0	43.9 ± 3.6	46.7 ± 1.9	0.82 ± 0.02	0.81 ± 0.03	8.1 ± 0.4	8.5 ± 1.1 a	4.3 ± 0.3	3.4 ± 0.4 a	258 ± 41	236 ± 23 a
	180	42.3 ± 1.6	43.9 ± 9.4	0.82 ± 0.03	0.82 ± 0.01	7.8 ± 0.3	6.1 ± 0.8b	4.0 ± 0.2	2.4 ± 0.3b	219 ± 42	115 ± 35b
4	0	46.1 ± 1.8	45.5 ± 1.4	0.81 ± 0.04	0.79 ± 0.06	9.1 ± 0.2 a	9.2 ± 0.7 a	4.4 ± 0.2 a	3.7 ± 0.3 a	246 ± 37 a	219 ± 29 a
	180	44.3 ± 2.4	46.5 ± 7.3	0.81 ± 0.02	0.80 ± 0.02	6.8 ± 1.0b	3.5 ± 1.4b	2.9 ± 0.5b	1.4 ± 0.6b	116 ± 24b	26 ± 8b
7	0	50.4 ± 2.3	49.3 ± 2.4	0.82 ± 0.03	0.80 ± 0.03	8.6 ± 0.8 a	8.3 ± 0.4 a	4.2 ± 0.3 a	3.3 ± 0.2 a	252 ± 46 a	224 ± 32 a
	180	50.0 ± 2.9	51.6 ± 7.8	0.81 ± 0.02	0.81 ± 0.01	5.7 ± 0.9b	2.8 ± 0.2b	2.6 ± 0.2b	1.8 ± 0.2b	98 ± 19b	39 ± 6b

[/1] Time represents the days after glyphosate application.
[/2] AE means acid equivalent of glyphosate.
[/3] SPAD index is correlated to leaf chlorophyll content.
[/4] Fv/Fm represents the maximum quantum efficiency of photosysthem II, calculated by taking the variable fluorescence (Fv) and dividing it by the maximum fluorescence (Fm).
[/5,/6,/7] are related to gas exchange, where: CO_2 flux represents the CO_2 assimilation rate (photosynthesis); H_2O flux represents the transpiration rate; GS represents the stomatal conductance; Different lowercase letters indicate significant differences between glyphosate doses in each time of evaluation by Tukey test at 5% probability of error.

Considering the biomass accumulation as a final response of survival plants to the herbicide application, the GG100 clone might be considered more susceptible to glyphosate field doses than the I144 clone, since shoot dry mass reduction was significantly higher in the GG100 clone (13%). On the other hand, the estimated glyphosate dose required to reduce the plant growth by 50% was found at 159 and 131 g AE ha^{-1} for the clones GG100 and I144, respectively, considering the average EC50 of all plant growth parameters, and the Hillslope regression parameter was higher in the I144 clone than the GG100 clone for all plant growth parameters (Table 1). In addition, the specific dose required to reduce shoot dry mass by 50% (134.2 g AE ha^{-1}) was 33% higher in the GG100 clone (Table 1). Thus, the GG100 clone might be considered less susceptible to glyphosate low doses than the I144 clone, so that an accidental drift of glyphosate would result in a more significant impact on the I144 clone.

These results document a different plant growth responses to glyphosate application between the two clones of eucalyptus, as previously found by other authors (Tuffi Santos et al., 2007b; Carvalho et al., 2015, 2018). Tuffi Santos et al. (2007b) found differences among species of eucalyptus and also between clones of eucalyptus. Carvalho et al. (2015) studied plant growth response of four clones of *Eucalyptus* × *urograndis* to glyphosate and found that the clones GG100 and I144 were the most susceptible to the herbicide. Carvalho et al. (2018) studied physiological responses of two clones of *Eucalyptus* × *urograndis* to glyphosate and found a strong reduction on gas exchange of the GG100 clone. As discussed by Carvalho et al. (2015), differences in the response of plants to the exposure to glyphosate can be derived from differences in spray retention or/and drop contact angle in the leaves, composition of the leaf epicuticular wax, herbicide absorption or/and translocation and herbicide degradation. Thus, any difference in morphological, physiological and metabolic traits may influence the response of the plant to glyphosate, resulting in greater or lesser susceptibility or tolerance to this herbicide.

Morphological changes due to exposure to glyphosate were previously found, although no difference in leaf morphology was observed that could explain the differential tolerance among clones of eucalyptus (Tuffi Santos et al., 2007b). In this study, we found that glyphosate applied at 180 g AE ha^{-1} caused changes in leaf morphology of the GG100 clone after 14 DAA, increasing stomatal index by 30%, whereas reducing nervure thickness by 17% at 30 DAA, with no effect on the I144 clone (Table 2). These results indicate that differences in plant responses to glyphosate occur between the two eucalyptus clones. However, there is no evidence that the morphological change might explain the differential plant response since morphological differences were observed only at 30 DAA (Table 2).

A low content of glyphosate (< 30 μg mg^{-1}) was detected, while AMPA was not detected in leaf tissues of both clones at the first week after application of 180 g AE ha^{-1} of this herbicide (Table 3). These results suggest that neither differences in herbicide absorption nor the herbicide degradation might explain the small differences in plant growth responses to glyphosate found between these clones of eucalyptus. However, the I144 clone showed significant glyphosate-caused accumulation of shikimic acid at 1 DAA, whereas a significant increase did not occur at this time in the GG100 clone (Table 3), indicating early effects on the shikimate pathway in the I144 clone. Therefore, these results suggest that glyphosate is translocated to its site of action faster in the I144 clone than in the GG100 clone, which may in part explain the different plant growth responses to glyphosate found

between clones of eucalyptus.

Shikimic acid accumulation has been used as a marker for EPSPS sensitivity in plants to glyphosate (González-Torralva et al., 2010) and also as an indicator of whether glyphosate is reaching the target enzyme (Powles and Preston, 2006). In general, the increase in shikimic acid level and accumulation has been found in association to the movement of glyphosate from the treated leaf into stem and roots, explaining the differential susceptibility among weed species (González-Torralva et al., 2010) or the resistance between weed biotypes to glyphosate (Carvalho et al., 2012; Alcántara-de la Cruz et al., 2016; Palma-Bautista et al., 2019). Thus, in response to inhibition of EPSPS caused by glyphosate, as previously discussed, the earlier accumulation of shikimic acid in the I144 clone is evidence that the herbicide may reach its site of action faster in this clone. If so, the shikimate pathway is blocked earlier in the I144 clone than the GG100 clone which probably influences the plant growth differently in a clone-dependent response.

We found no effect of glyphosate on SPAD index and Fv/Fm (Table 4), indicating neither the chlorophyll content nor the quantum efficiency of photosystem II was affected by glyphosate application in both clones of eucalyptus. Carvalho et al. (2016) did not observe either reduction in chlorophyll content and Fv/Fm of eucalyptus treated with up to 180 g AE ha^{-1} of glyphosate, but a reduction in chlorophyll content was found in eucalyptus treated with doses $\geq$ 360 g AE ha^{-1}. Olesen and Cedergreen (2010) did not also found any change in Fv/Fm of barley exposed to glyphosate doses $<$ 720 g AE ha^{-1}, but fluorescence measurements on plants sprayed with 720 g AE ha^{-1} could not be made later than at 4 DAA, as the plants had lost turgor and the leaves were collapsing and curling. These results suggest that photochemical reactions of photosynthesis might not be significantly affected by the low doses of glyphosate in this study. However, caution should be taken in the use of chlorophyll fluorescence as a universal indicator of stress on photosynthetic processes (Olesen and Cedergreen, 2010).

On the other hand, we found that glyphosate reduced the CO_2 assimilation rate, transpiration rate and stomatal conductance in both clones of eucalyptus at the first week after application of 180 g AE ha^{-1} of glyphosate (Table 4). Both the CO_2 influx and the H_2O efflux occur through the stomata, so that the stomatal movement is the key mechanism for controlling gas exchange, except in primitive plants (Nascentes et al., 2018). Photosynthesis depends on the flow of CO_2 into the cell, and, in turn, the CO_2 flow depends on the stomatal opening (Messinger et al., 2006). The process of controlling gas exchange causes plants to maintain a stomatal aperture that avoids water stress while maximizing carbon fixation with the balance between CO_2 uptake and water loss (Nascentes et al., 2018). Therefore, the stomatal closure caused by glyphosate can be the cause of the reduced rates of photosynthesis and transpiration. Alterations in gas exchange caused by glyphosate were previously found in eucalyptus (Carvalho et al., 2018; Nascentes et al., 2018), indicating that biochemical reactions of photosynthesis may be significantly affected by glyphosate.

Moreover, the reduction found on gas exchange started earlier in the I144 clone (1 DAA) than in the GG100 clone (4 DAA), indicating that alterations in gas exchange may in part explain the small differences in plant growth responses to glyphosate found between clones of eucalyptus in this study. We hypothesize that early differences in gas exchange might persist during a period after application of low glyphosate doses, impacting the plant growth of the two clones differently. Differences in gas exchange between clones of eucalyptus were previously observed (Carvalho et al., 2018). However, this is the first report that the differential plant growth response between clones of eucalyptus to glyphosate may be associated with a differential photosynthetic response.

5. Conclusion

The magnitude of the effect of glyphosate herbicide on the early plant growth and leaf morphology of *Eucalyptus* × *urograndis* is dependent on the species genotype (clone). This clone-dependent response may be due in part to differences in both herbicide mode of action-related plant metabolism and gas exchange. Differences in susceptability to glyphosate by closely related clones is unlikely to be due to differences in one plant property.

CRediT authorship contribution statement

Wilson Roberto Cerveira Junior: Investigation. **Yanna Karoline Santos da Costa:** Investigation. **Caio Antonio Carbonari:** Methodology. **Stephen Oscar Duke:** Writing - original draft, Writing - review & editing. **Pedro Luis da Costa Aguiar Alves:** Conceptualization, Supervision. **Leonardo Bianco de Carvalho:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alcántara-de la Cruz, R., Rojano-Delgado, A.M., Giménez, M.J., Cruz-Hipolito, H.E., Domínguez-Valenzuela, J.A., Barro, F., De Prado, R., 2016. First resistance mechanisms characterization in glyphosate-resistant Leptochloa virgata. Front. Plant Sci. 7, 1742.

Amaral, E.S., Silva, D.V., Dos Anjos, L., Schilling, A.C., Dalmolin, A.C., Mielke, M.S., 2019. Relationships between reflectance and absorbance chlorophyll indices with RGB (Red, Green, Blue) image components in seedlings of tropical tree species at nursery stage. New Forest. 50, 377–388.

Assis, T.F., Abad, J.I.M., Aguiar, A.M., 2015. Melhoramento Genético do Eucalipto. In: Schumacher, M.V., Viera, M. (Eds.), Silvicultura do Eucalipto no Brasil. UFSM, Santa Maria.

Bassaco, M.V.M., Motta, A.C.V., Pauletti, V., Prior, S.A., Nisgoski, S., Ferreira, C.F., 2018. Nitrogen, phosphorus, and potassium requirements for *Eucalyptus urograndis* plantations in southern Brazil. New Forest. 49, 681–697.

Becerril, J.M., Duke, S.O., Lydon, J., 1989. Glyphosate effects on shikimate pathway products in leaves and flowers of velvetleaf. Phytochemistry 28, 695–699.

Belz, R.G., Duke, S.O., 2014. Herbicides and plant hormesis: Herbicides and plant hormesis. Pest. Manag. Sci. 70 (5), 698–707.

Boudet, A.M., Graziana, A., Ranjeva, R., 1985. Recent advances in the regulation of the prearomatic pathway. In: Van Sumere, C.F., Lea, P.J. (Eds.), Annual Proceedings of the Phytochemistry Society Europe. Clarendon Press, Oxford, pp. 135–159.

Carvalho, L.B., Alves, P.L.C.A., González-Torralva, F., Cruz-Hipolito, H.E., Rojano-Delgado, A.M., De Prado, R., 2012. Pool of resistance mechanisms to glyphosate in *Digitaria insularis*. J. Agric. Food. Chem. 60, 615–622.

Carvalho, L.B., Alves, P.L.C.A., Duke, S.O., 2013. Hormesis with glyphosate depends on coffee growth stage. Ann. Braz. Acad. Sci. 85, 813–821.

Carvalho, L.B., Alves, P.L.C.A., Costa, F.R., 2015. Differential response of clones of eucalypt to glyphosate. Revista Árvore 39, 177–187.

Carvalho, L.B., Alves, P.L.C.A., Andrade, T.C.G.R., 2016. Content and fluorescence of chlorophyll in eucalypt exposed to glyphosate. Commun. Plant Sci. 6, 7–11.

Carvalho, L.B., Duke, S.O., Alves, P.L.C.A., 2018. Physiological responses of *Eucalyptus* x *urograndis* to glyphosate are dependent on the genotype. Scientia Forestalis 46, 177–187.

Cedergreen, N., Olesen, C.F., 2010. Can glyphosate stimulate photosynthesis? Pestic. Biochem. Physiol. 96, 140–148.

Costa, A.C.P.R., Costa, N.V., Pereira, M.R.R., Martins, D., 2012. Simulated drift effect of glyphosate in different parts of *Eucalyptus grandis* plants. Semina. Ciências Agrária 33, 1663–1672.

Cruz-Hipólito, H., Osuna, M.D., Heredia, A., Ruiz-Santaella, J.P., De Prado, R., 2009. Non target mechanism involved in glyphosate tolerance found in Canavalia ensiformis

plants. J. Agric. Food. Chem. 57, 4844–4848.
Duke, S.O., 2011. Glyphosate degradation in glyphosate-resistant and -susceptible crops and weeds. J. Agric. Food. Chem. 59, 5835–5841.
Duke, S.O., Powles, S.B., 2008. Glyphosate: a once-in-a-century herbicide. Pest Manag. Sci. 64, 319–325.
Duke, S.O., 2019. Enhanced metabolic degradation of glyphosate: The last evolved resistance mechanism? Plant Physiol. 181, 1401–1403.
Fisher, R.S., Berry, A., Gaines, C.G., Jensen, R.A., 1986. Comparative action of glyphosate as a trigger of energy drain in *Eubacteria*. J. Bacteriol. 168, 1147–1154.
Florentine, S., Fox, J., 2003. Competition between *Eucalyptus victrix* seedlings and grass species. Ecol. Res. 18, 25–39.
Franz, J.E., Mao, M.K., Sikorski, J.A., 1997. Uptake, transport and metabolism of glyphosate in plants. In: Franz, F.E., Mao, M.K., Sikorski, J.A. (Eds.), Glyphosate: a unique global herbicide. American Chemical Society, Washington DC, pp. 143–181.
Garau, A.M., Lemcoff, J.H., Ghersa, C.M., Barañao, J.J., 2009. Weeds in *Eucalyptus globulus* subsp. *maidenii* (F. Muell) establishment: effects of competition on sapling growth and survivorship. New Forests 37, 251–264.
Ge, X., D'Avignon, D.A., Ackermana, J.J.H., Sammons, R.D., 2010. Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. Pest Manag. Sci. 66, 345–348.
Ge, X., D'Avignon, D.A., Ackerman, J.J., Collavo, A., Sattin, M., Ostrander, E.L., Hall, E.L., Sammons, R.D., Preston, C., 2012. Vacuolar glyphosate-sequestration correlates with glyphosate resistance in ryegrass (*Lolium* spp.) from Australia, South America, and Europe: a 31P NMR investigation. J. Agric. Food. Chem. 60, 1243–1250.
Gomes, G.L.G.C., Carbonari, C.A., Velini, E.D., Trindade, M.L.B., Silva, J.R.M., 2015. Extraction and simultaneous determination of glyphosate, AMPA and compounds of the shikimic acid pathway in plants. Planta Daninha 33, 295–304.
Gomes, M.P., Le Manac'h, S.G., Maccario, S., Labrecque, M., Luccote, M., Juneau, P., 2016. Differential effects of glyphosate and aminomethylphosphonic acid (AMPA) on photosynthesis and chlorophyll metabolism in willow plants. Pesticide Biochem. Physiol. 130, 65–70.
Gomes, M.P., Le Manc'h, S.G., Hénault-Ethier, L., Labrecque, M., Luccote, M., Juneau, P., 2017. Glyphosate-dependent inhibition of photosynthesis in willow. Front. Plant Sci. 8, 207.
González-Torralva, F., Cruz-Hipolito, H., Bastida, F., Mülleder, N., Smeda, R.J., De Prado, R., 2010. Differential susceptibility to glyphosate among the *Conyza* weed species in Spain. J. Agric. Food. Chem. 58, 4361–4366.
Gruys, K.J., Sikorski, J.A., 1999. Inhibitors of tryptophan, phenylalanine and tyrosine biosynthesis as herbicides. In: Singh, B.K. (Ed.), Plant amino acids: biochemistry and biotechnology. Marcel Dekker, New York, pp. 357–384.
Harwood, C., 2011. New introductions – doing it right. In: Walker, J. (Ed.), Developing a eucalypt resource. Learning from Australia and elsewhere. Wood Technology Research Centre, University of Canterbury, Christchurch, pp. 125–136.
Herrmann, K.M., 1995. The shikimate pathway: early steps in the biosynthesis of aromatic compounds. Plant Cell 7, 907–919.
Hoagland, D., Arnon, D.I., 1950. The water-culture method for growing plants without soil. AES, California, pp. 32.
Johansen, D.A., 1940. Plant microtechnique. McGraw-Hill, New York, pp. 128.
Krauter, D., 1985. Erfahrungen mit Etzolds FSA-Färbung für pflanzenschnitte. Mikrokosmos 74, 231–233.
Lydon, J., Duke, S.O., 1988. Glyphosate induction of elevated levels of hydroxybenzoic acids in higher plants. J. Agric. Food. Chem. 36, 813–818.
Machado, A.F.L., Ferreira, L.R., Santos, L.D.T., Ferreira, F.A., Viana, R.G., Machado, M.S., Freitas, F.C.L., 2010. Photosynthetic efficiency and water use in eucalyptus plants sprayed with glyphosate. Planta Daninha 28, 319–327.
Messinger, S.M., Buckley, T.N., Mott, K.A., 2006. Evidence for involvement of photosynthetic processes in the stomatal response to CO_2. Plant Physiol. 140, 771–778.
Nambiar, E.K.S., Sands, R., 1993. Competition for water and nutrients in forests. Can. J. For. Res. 23, 1955–1968.
Nascentes, R.F., Fagan, E.B., Soares, L.H., Oliveira, L.H., Oliveira, C.B., Brunelli, M.C., 2015. Hormesis de glyphosate em *Brachiaria brizantha* cv. Marandu. Cerrado Agrociências 6, 55–64.
Nascentes, R.F., Carbonari, C.A., Simões, P.S., Brunelli, M.C., Velini, E.D., Duke, S.O., 2018. Low doses of glyphosate enhance growth, CO_2 assimilation, stomatal conductance and transpiration in sugarcane and eucalyptus. Pest Manag. Sci. 74, 1197–1205.
Olesen, C.F., Cedergreen, N., 2010. Glyphosate uncouples gas exchange and chlorophyll fluorescence. Pest Manag. Sci. 66, 536–542.
Palma-Bautista, C., Alcántara-de la Cruz, R., Rojano-Delgado, A.M., Dellaferrera, I., Domínguez-Martínez, P.A., De Prado, R., 2019. Low temperatures enhance the absorption and translocation of 14C-glyphosate in glyphosate-resistant Conyza sumatrensis. J. Plant Physiol. 240, 153009.
Pan, L., Yu, Q., Han, H., Mao, L., Nyporko, A., Fan, L., Bai, L., Powles, S.B., 2019. Aldo-keto reductase metabolizes glyphosate and confers glyphosate resistance in *Echinochloa colona*. Plant Physiol. 181, 1520–1534.
Potts, B.M., Dungey, H.S., 2004. Hybridization of eucalyptus: key issues for breeders and geneticists. New Forest. 27, 115–138.
Powles, S.B., Preston, C., 2006. Evolved glyphosate resistance in plants: biochemical and genetic basis of resistance. Weed Technol. 20, 282–289.
Preston, C., Wakelin, A.M., 2008. Resistance to glyphosate from altered herbicide translocation patterns. Pest Manag. Sci. 64, 372–376.
Rojano-Delgado, A.M., Ruiz-Jiménez, J., Castro, M.D.L., De Prado, R., 2010. Determination of glyphosate and its metabolites in plant material by reversed-polarity CE with indirect absorptiometric detection. Electrophoresis 31, 1423–1430.
Sakaigaichi, T., Tsuchida, H., Adachi, K., Hattori, T., Tarumoto, Y., Tanaka, M., Hayano, M., Sakagami, J.I., Irei, S., 2019. Phenological changes in the chlorophyll content and its fluorescence in field-grown sugarcane clones under over-wintering conditions. Sugar Tech 21 (843), 846.
Salgado, T.P., Alves, P.L.C.A., Kuva, M.A., Takahashi, E.M., Dias, T.C.S., Lemes, L.N., 2011. Initial symptoms of *Eucalyptus* intoxication by glyphosate rates applied on the stem or leaves. Planta Daninha 29, 913–922.
Salgado, T.P., Pereira, F.M.C., Kuva, M.A., Alves, P.L.C.A., 2017. Effects of glyphosate on growth, yield and wood quality of *Eucalyptus urograndis*. J. Trop. For. Sci. 29, 257–266.
Salisbury, E.J., 1927. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. Philosoph. Trans. London 216, 1–65.
Silva, P.H.M., Junqueira, L.R., Araujo, M.J., Wilcken, C.F., Moraes, M.L.T., Paula, R.C., 2019. Susceptibility of eucalypt taxa to a natural infestation by *Leptocybe invasa*. New Forests. https://doi.org/10.1007/s11056-019-09758-1. (in press).
Simetti, R., Bonduelle, G.M., Silva, D.A., Mayer, S.L.S., Souza, H.P., Muniz, G.I.B., 2018. Production of biomass and energy stock for five *Eucalyptus* species. Brazilian J. Wood Sci. 9, 30–36.
Tuffi Santos, L.D., Ferreira, F.A., Meira, R.M.S.A., Barros, N.F., Ferreira, L.R., Machado, A.F.L., 2005. Crescimento e morfoanatomia foliar de eucalipto sob efeito de deriva do glyphosate. Planta Daninha 23, 133–142.
Tuffi Santos, L.D., Machado, A.F.L., Viana, R.G., Ferreira, L.R., Ferreira, F.A., Souza, G.V.R., 2007a. Crescimento do eucalipto sob efeito da deriva de glyphosate. Planta Daninha 25, 133–137.
Tuffi Santos, L.D., Meira, R.M.S.A., Ferreira, F.A., Sant'Anna-Santos, B.F., Ferreira, L.B., 2007b. Morphological responses of different eucalypt clones submitted to glyphosate drift. Environ. Experim. Botany 59, 11–20.
Tuffi Santos, L.D., Sant'Anna-Santos, B.F., Meira, R.M.S.A., Ferreira, F.A., Tiburcio, R.A.S., Machado, A.F.L., 2009. Leaf anatomy and morphometry in three eucalypt clones treated with glyphosate. Brazilian J. Biol. 69, 129–136.
Velini, E.D., Alves, E., Godoy, M.C., Meschede, D.K., Souza, R.T., Duke, S.O., 2008. Glyphosate applied at low doses can stimulate plant growth. Pest Manag. Sci. 64, 489–496.