DIFFERENTIAL RESPONSE OF CLONES OF EUCALYPT TO GLYPHOSATE[1]

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ABSTRACT – Weed control is commonly performed by the inter-row mechanical weeding associated to intra-row glyphosate directed spraying, causing a risk for drift or accidental herbicide application, that can affect the crop of interest. The objective was to evaluate the response of clones C219, GG100, I144, and I224 of eucalypt (*Eucalyptus grandis* x *E. urophylla*) to glyphosate doses of 0, 18, 36, 72, 180, 360, and 720 g of acid equivalent per hectare. The clones showed different growth patterns with regard to height, leaf number, stem dry weight, relative growth rate, net assimilation rate, and relative leaf growth rate. The clones I144 and GG100 were more susceptible to glyphosate, showing the doses required to reduce dry weight by 50% of 113.4 and 119.6 g acid equivalent per hectare, respectively. The clones C219 and I224 were less susceptible to glyphosate, showing the doses required to reduce dry weight by 50% of 237.5 and 313.5 g acid equivalent per hectare, respectively. Eucalyptus clones respond differently to glyphosate exposure, so that among I224, C219, GG100, and I144, the susceptibility to the herbicide is increasing.

Keywords: *Eucalyptus*; *N*-(phosphonomethyl)-glycine; Spray drift.

RESPOSTA DIFERENCIAL DE CLONES DE EUCALIPTO A GLYPHOSATE

*RESUMO – O controle de plantas daninhas é comumente feito com roçadas na entrelinha associada a aplicações dirigidas de glyphosate na linha de plantio de culturas arbóreas, acarretando risco de deriva ou aplicação acidental do herbicida, que pode afetar a cultura de interesse. O objetivo foi avaliar a resposta dos clones C219, GG100, I144 e I224 de eucalipto (**Eucalyptus grandis** x **E. urophylla**) a doses de glyphosate de 0, 18, 36, 72, 180, 360 e 720 g de equivalente ácido por hectare. Os clones apresentaram padrões de crescimento distintos no que se refere a altura da planta, número de folhas, massa seca do caule, taxa de crescimento relativo, taxa de assimilação líquida e taxa de crescimento foliar relativo. Os clones I144 e GG100 foram mais suscetíveis ao glyphosate, sendo as doses necessárias para reduzir a massa seca em 50% de 113,4 e 119,6 g ea ha^{-1}, respectivamente. Os clones C219 e I224 foram menos suscetíveis ao glyphosate, sendo as doses necessárias para reduzir a massa seca em 50% de 237,5 e 313,5 gae ha^{-1}, respectivamente. Clones de eucalipto respondem diferentemente à exposição ao glyphosate, e entre I224, C219, GG100 e I144 a suscetibilidade é aumentada.*

*Palavras-chave: **Eucalyptus**; N-(fosfonometil)-glicina; Deriva.*

1. INTRODUCTION

Forest species of the genus *Eucalyptus* have high potential for wood production due to their biodiversity, environmental adaptability, high productivity, and excellent wood physic-chemical characteristics, allowing the eucalypt to show many uses as a wood-based raw material. However, one of the main issues in eucalypt plantations is related to the presence of weeds interfering with the crop, so that the weed management assumes an outstanding role as a cultural practice, showing direct reflexes on the yield and production costs (TUFFI SANTOS, 2006). Weed control in perennial plantations

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have been commonly performed by the inter-row mechanical weeding associated to the intra-row glyphosate directed spraying (CARVALHO et al., 2012a), causing a risk for spray drift if the herbicide is sprayed under inadequate environmental conditions or/and the application technology is misused (MACHADO et al., 2010).

The spray drift is defined as the transport of small drops (formed during the pesticide spraying) into the out of the target area (soil or/and leaves for herbicides), constituting in one of the main causes of herbicide losses in field conditions (COSTA et al., 2012b). Beyond the reduction in the weed control efficacy, the spray drift becomes more undesirable due mainly to the toxic effects to the crops of interest (YAMASHITA; GUIMARÃES, 2006; FIGUEREDO et al., 2007; COSTA et al., 2009; MACIEL et al., 2009; GUSMÃO et al., 2011) and the direct injury to sensible neighboring crops, increasing the financial losses by judicial actions (COSTA et al., 2012b). In addition, herbicide drift may contaminate the food, the air, the soil, and the water resources as well as it may cause detrimental effects on human and animal security and health (COSTA et al., 2012b).

The glyphosate [*N*-(phosphonomethyl) glycine] is a non-selective systemic herbicide used during the past 35 years. It is the most important herbicide worldwide due to its versatility of using in agricultural and forest areas. The glyphosate is one of the main herbicides used for weed control in eucalypt plantations (COSTA et al., 2012a) due to a few products are officially registered and due in addition to some favorable characteristics of the glyphosate (TUFFI SANTOS et al., 2007), 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; WAKELIN, 2008).

Showing a large spectrum and being a non-selective herbicide, the glyphosate drift may cause deleterious effects on the growth, development, and production, or even kill plants of the crops of interest (CARVALHO et al., 2012a). However, a different behavior may be observed among distinct plants of the same species when they are exposed to the glyphosate drift. Thus, the objective of this research was to evaluate the growth pattern of four clones of *Eucalyptus grandis* x *Eucaliptus urophylla* (*Eucalyptus urograndis*), known as C219 (commonly cultivated), GG100, I144, and I224 (of recent use in Brazil and with no information in the literature), submitted to glyphosate exposure at different doses.

2. MATERIAL AND METHODS

Controlled experiments were carried out in Jaboticabal, SP, Brazil (21º15'22" latitude S and 48º18'58" longitude W) in 2011. Plants grew in a growth chamber under the temperature of 25±2 ºC, the photoperiod of 14:10 h (light:dark), and the photosynthetically active radiation of 400 µmol m^{-2} s^{-1} delivered by fluorescence lights.

Eucalypt plantlets (obtained from vegetative propagation in clonal minigardens of Fibria, Brazil) were planted into 3-L pots filled with a mixture of organic substrate and watered river sand in a proportion of 1:1 (v:v). Pots were daily irrigated with 100 mL of the nutrient solution of Hoagland and Arnon (1950) at 50% of the original concentration. After 25 days after planting, 50 mL of distilled water was also supplied in addition to the nutrient solution due to the plant growth stage.

Treatments consisted of a factorial scheme 4×7 (four eucalypt clones and six glyphosate doses plus a non-treated check for each clone), arranged in a completely randomized design with six replicates. Glyphosate (Roundup Original®, Monsanto, Brazil) was sprayed at doses of 18, 36, 72, 180, 360, and 720 g of acid equivalent per hectare (g ae ha^{-1}),directly onto the eucalypt shoot by using a CO_2 backpack-sprayer equipped with four flat fan nozzles (110:02, Tee Jet, Brazil) at 2 bar pressure and 200 L ha^{-1} spray volume. In addition, water was sprayed with regards to the non-treated check (0 g ae ha^{-1} of glyphosate). Glyphosate spraying was performed at 50 cm from the top of the plants after the eucalypt plantlets were kept in a period of acclimation during 10 days within the growth chamber.

Growth characteristics, such as plant height, stem diameter, leaf number, leaf area, leaf dry mass, and stem dry mass were evaluated at 30 days after glyphosate application (DAA) (Time 2). The same characteristics were also evaluated in samples of six individuals of each clone before glyphosate spraying (Time 1). Plant height and stem diameter was measured by using a graduated yardstick (0.1 cm) and a digital caliper (0.01 cm), respectively. Leaf area was determined by using a leaf area meter (Li-Cor Inc., LI3000A, USA) (0.01 cm^2). Leaf dry mass and stem dry mass were weighted by using a semi-analytical balance (0.01 g), after drying the plant material in a forced air-convection oven at 60±5 ºC during 96 h.

Physiological plant growth indexes were calculated according to Peixoto and Peixoto (2009): absolute growth rate (AGR), relative growth rate (RGR), leaf area ratio (LAR), specific leaf area (SLA), leaf mass ratio (LMR), net assimilation rate (NAR), relative leaf growth rate (RLGR), crop growth rate (CGR), and leaf area index (LAI). The formulas were:

$$AGR = \frac{\Delta DMt}{\Delta T}\ (g day^{-1})$$

$$RGR = \frac{\Delta lnDMt}{\Delta T}\ (g\ g^{-1} day^{-1})$$

$$LAR = \frac{LA}{DMt}\ (cm^2 g^{-1})$$

$$SLA = \frac{LA}{DMf}\ (cm^2 g^{-1})$$

$$LMR = \frac{DMf}{DMt}\ (g\ g^{-1})$$

$$NAR = \frac{\Delta DMt\ \times\ \Delta lnLA}{\Delta LA\ \times\ \Delta T}\ (g\ cm^{-2} day^{-1})$$

$$LRGR = \frac{\Delta lnLA}{\Delta T}\ (cm^2 cm^{-2} day^{-1})$$

$$CGR = \frac{\frac{\Delta DMt}{A}}{\Delta T}\ (g\ m^{-2} day^{-1})$$

$$LAI = \frac{AF}{A}\ (cm^2 m^{-2})$$

where: DMt, T, LA, DMf, and A indicate the shoot dry mass, the time of growing, the leaf area, the leaf dry mass, and the pot superficial area. The A indicates a variation of both plant growth characteristic and time between the times of evaluation Time 2 and Time 1 (as described above).

Data were submitted to ANOVA (F test) in a 4×7 factorial scheme (four clones and seven doses) by using the software Statistica (version 8.0, StatSoft, USA). Dry mass data were also submitted to a non-linear regression analysis by using the software Sigma Plot (version 10.0, Systat, USA) to establish a relation between the plant dry mass accumulation and the exposure of increasing doses of glyphosate. We used a non-linear model as follows:

$$y = c + \frac{d - c}{1 + \left(\frac{x}{g}\right)^b}$$

where: y indicates the dry mass value, c and d are curve coefficients indicating the minimum and the maximum dry mass value, b is the slope of the curve, g is the point of inflexion of the curve, representing the dose required to reduce the dry mass accumulation by 50% (GR50), and x indicates the glyphosate dose.

We also calculated the relative differential susceptibility (RDS) among the four clones, according to the formula:

$$RDS = \frac{GR50_H}{GR50_L}$$

where: $GR50_L$ indicates the lowest GR50 of a specific clone and $GR50_H$ indicates higher GR50 (GR50 of the other clones), so that the higher RDS, the less susceptibility to glyphosate.

3. RESULTS

There was significant interactions between the factors clones and doses for plant height ($P = 0.018$), leaf number ($P = 0.006$), stem dry mass ($P = 0.003$), relative growth rate ($P = 0.042$), net assimilation rate ($P<0.001$), and relative leaf growth rate ($P<0.001$), indicating that, for those characteristics, at least one of the clones showed different dose-response to the glyphosate when compared with the other ones. That difference among the eucalypt clones was stronger at low doses of glyphosate for plant height and leaf number (Figures 1 and 2), while differences occurred at high doses of glyphosate for stem dry mass, net assimilation rate, and relative leaf growth rate (Figures 1 and 2). However, for relative growth rate, differences were observed at low doses and at the highest dose (Figure 2).

For the other characteristics, there was an isolated significant effect of the factor clone on the stem diameter ($P<0.001$), the leaf area ($P = 0.020$), the shoot dry mass ($P = 0.006$), the absolute growth rate ($P = 0.006$), the leaf area ratio ($P=0.002$), the specific leaf area ($P=0.002$), the crop growth rate ($P = 0.006$), and the leaf area index ($P=0.020$); and also the factor dose ($P<0.001$) on all characteristics. The results indicated that, for those characteristics, there were differences among the eucalypt clones but the dose-response to glyphosate was similar. Those differences may be attributed to

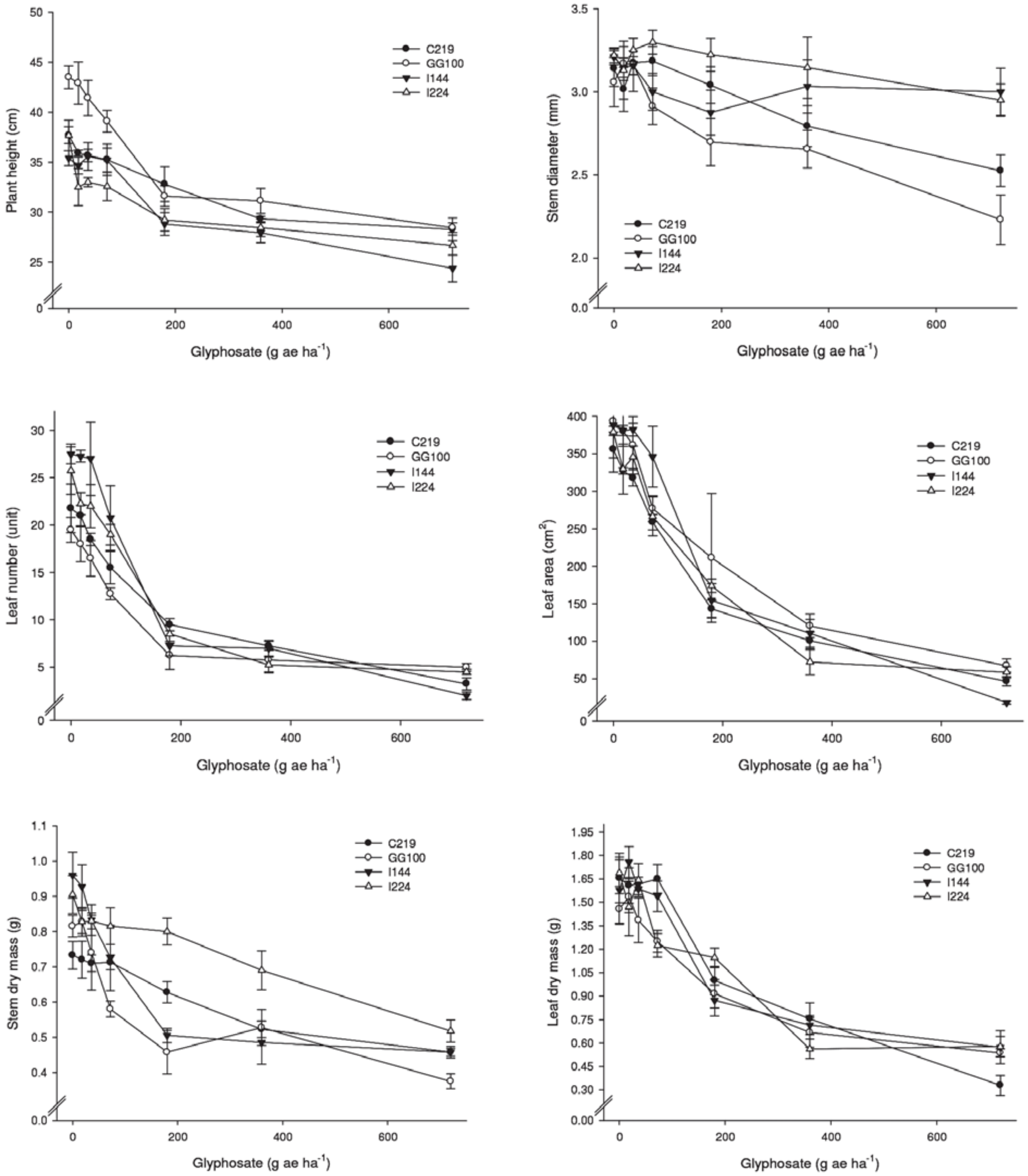

Figure 1 – Growth characteristics of four clones of eucalyptus (*Eucalyptus grandis* x *Eucalyptus urophylla*) at thirty days after being exposed to different doses of glyphosate. Vertical lines indicate the standard error of the mean of six replicates.

Figura 1 – *Características de crescimento de quatro clones de eucalipto (**Eucalyptus grandis** x **Eucalyptus urophylla**) aos 30 dias após a exposição a diferentes doses de glifosato. Linhas verticais indicam o erro-padrão da média de seis repetições.*

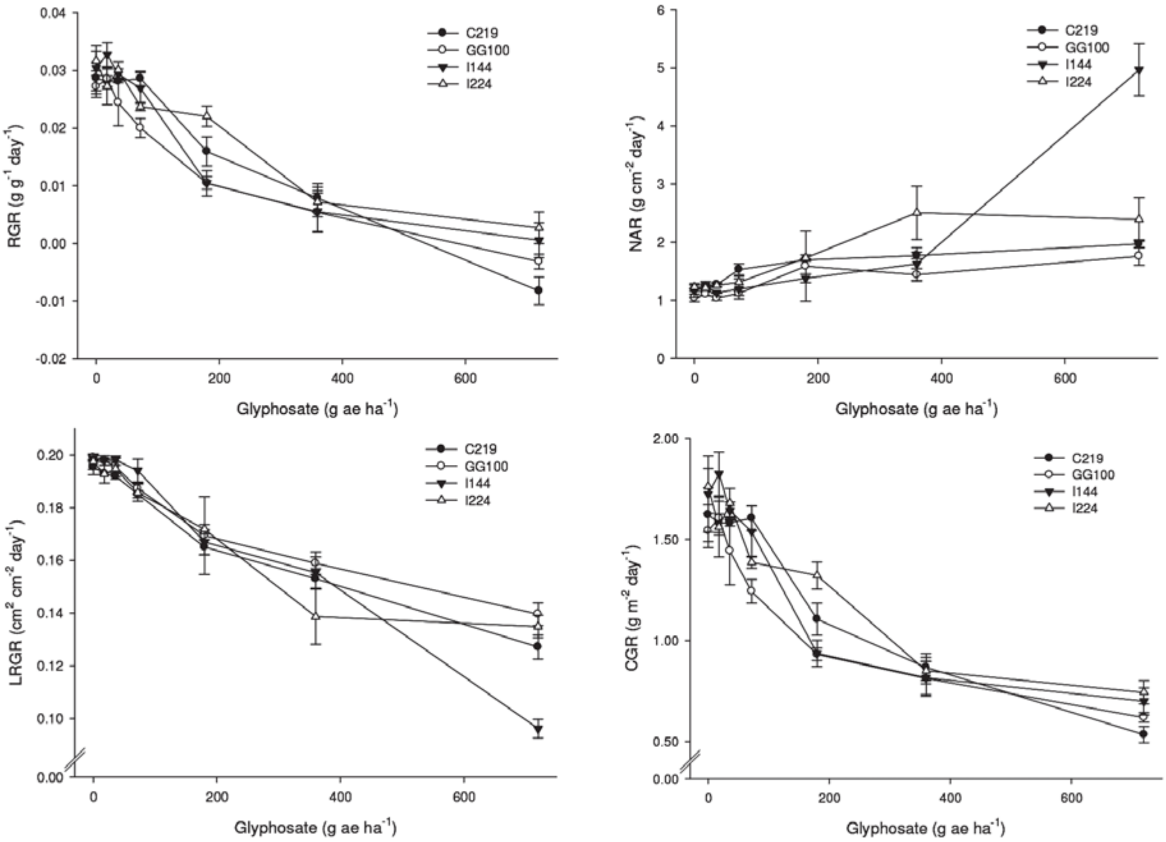

Figure 2 – Physiological plant growth indexes of four clones of eucalyptus (*Eucalyptus grandis* x *Eucalyptus urophylla*) exposed to different doses of glyphosate. Relative growth rate (RGR); net assimilation rate (NAR); relative leaf growth rate (RLGR); and crop growth rate (CGR). Vertical lines indicate the standard error of the mean of six replicates.

Figura 2 – *Índices fisiológicos de crescimento de plantas de quatro clones de eucalipto (**Eucalyptus grandis** x **Eucalyptus urophylla**) expostos a diferentes doses de glifosato. Taxa de crescimento relativo (RGR); taxa de assimilação líquida (NAR); taxa de crescimento foliar relativo (RLGR); e taxa de crescimento da cultura (CGR). Linhas verticais indicam o erro-padrão da média de seis repetições.*

genetic factors, being not an exclusive consequence of the glyphosate exposure. Strong differences occurred on the stem diameter, the leaf area ratio, the specific leaf area, and the leaf area index, even at low doses of glyphosate (Figures 1 and 3). For the leaf area, the absolute growth rate, and the crop growth rate, just isolated differences were verified among the clones at both low and high doses (Figures 1 and 2).

In addition, there was an isolated significant effect just of the factor dose ($P<0.001$) on the leaf dry mass (Figure 1), indicating that the leaf dry mass was similar for all clones and the clones also responded similarly to the glyphosate exposure.

The analysis of plant dry mass accumulation revealed that the adjusted regression model was significant for all clones of eucalypt (Table 1). Significant differences were observed in the response of the four clones of eucalypt to the increase in the glyphosate dose (Figure 4). The clones I144 (GR50 of 113.4 g ae ha^{-1}) and GG100 (GR50 of 119.6 g ae ha^{-1}) were more susceptible than the clones C219 (GR50 of 237.5 g ae ha^{-1}) and I224 (GR50 of 313.5 gae ha^{-1}) (Table 1).

4. DISCUSSION

The plant growth reduction was verified by analyzing the growth characteristics, as plant height, stem diameter,

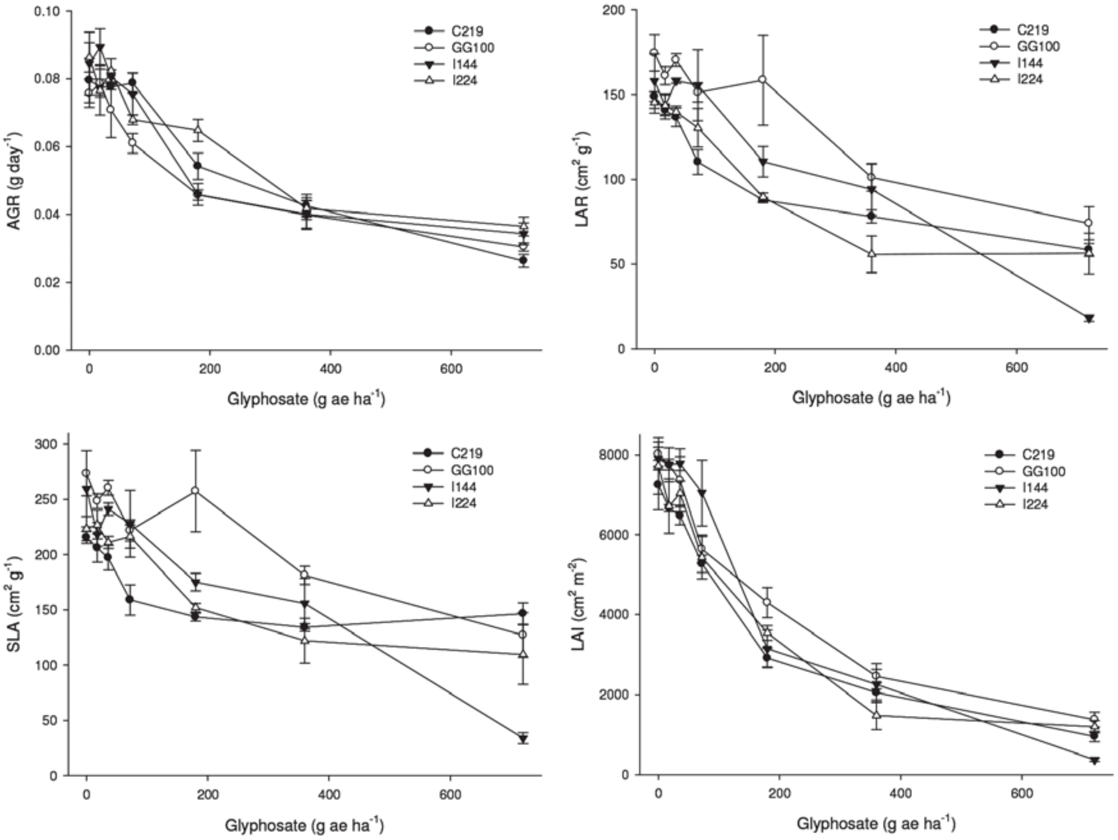

Figure 3 – Physiological plant growth indexes of four clones of eucalyptus (*Eucalyptus grandis* x *Eucalyptus urophylla*) exposed to different doses of glyphosate. Absolute growth rate (AGR); leaf area ratio (LAR); specific leaf area (SLA); and leaf area index (LAI). Vertical lines indicate the standard error of the mean of six replicates.

***Figura 3** – Índices fisiológicos de crescimento de plantas de quatro clones de eucalipto (**Eucalyptus grandis** x **Eucalyptus urophylla**) expostos a diferentes doses de glifosato. Taxa de crescimento absoluto (AGR); razão de área foliar (LAR); área foliar específica (SLA); e índice de área foliar (LAI). Linhas verticais indicam o erro-padrão da média de seis repetições.*

Table 1 – Parameters of the regression equation of the shoot dry weight data of four clones of eucalyptus (*Eucalyptus grandis* x *Eucalyptus urophylla*) at thirty days after being exposed to different doses of glyphosate.

***Tabela 1** – Parâmetros da equação de regressão dos dados de massa seca da parte aérea de quatro clones de eucalipto (**Eucalyptus grandis** x **Eucalyptus urophylla**) aos 30 dias após a exposição a diferentes doses de glifosato.*

Clone	c	d	b	g	R^2	RDS
I144	1.06±0.10	2.59±0.07	2.60±0.68	113.4±15.9	0.978**	1.0
GG100	0.87±0.17	2.34±0.09	1.49±0.44	119.6±29.8	0.971**	1.1
C219	0.63±0.33	2.39±0.08	1.89±0.76	239.5±63.7	0.965**	2.1
I224	0.42±0.28	2.54±0.18	1.03±0.73	313.5±72.1	0.893*	2.8

Mean ± the standard error of the mean of six replicates.
Equation $y = c + \{(d–c)/[1 + (x/g)^b]\}$, where y indicates the dry weight value, c and d are curve coefficients indicating the minimum and the maximum dry weight value, b is the slope of the curve, g is the point of inflexion of the curve, representing the dose required to reduce the dry weight accumulation by 50% (GR50), and x indicates the glyphosate dose.
R^2 is the value of the adjusted coefficient of determination of the curve.
** and * indicate the significance of the adjusted regression model at 1% and 5% of probability by the F test.
RDS indicates the relative differential susceptibility between clones, referring to the ratio between the GR50 of the clones GG100, C219, or I224 and the lowest GR50 (of the clone I144), so that the higher the RDS, the smaller the susceptibility to glyphosate.

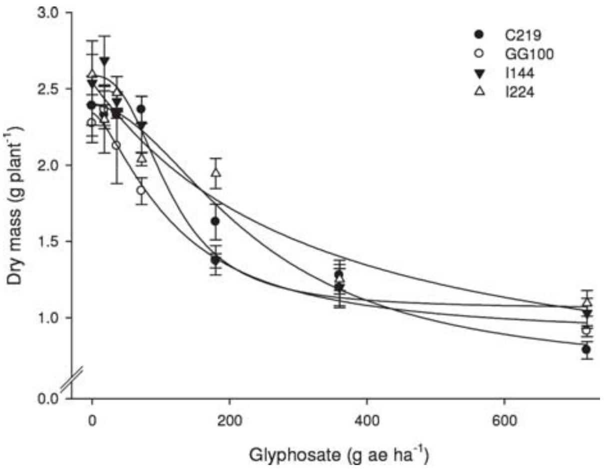

Figure 4 – Shoot dry weight of four clones of eucalyptus (*Eucalyptus grandis* x *Eucalyptus urophylla*) at thirty days after being exposed to different doses of glyphosate. Vertical lines indicate the standard error of the mean of six replicates.

Figura 4 – *Massa seca da parte aérea de quatro clones de eucalipto (**Eucalyptus grandis** x **Eucalyptus urophylla**) aos 30 dias após a exposição a diferentes doses de glifosato. Linhas verticais indicam o erro-padrão da média de seis repetições.*

leaf number, leaf area, leaf dry mass, and stem dry mass, that decreased by 30%, 15%, 84%, 87%, 68%, and 47%, respectively; and then it influenced the physiological indexes, as RGR, LRGR, CGR, AGR, LAR, SLA, and LAI, that reduced by 107%, 37%, 61%, 61%, 67%, 57%, and 87%, respectively, considering the average of the four clones under glyphosate application at the highest dose. On the other hand, NAR increased just by 40%, being expected a greater percentage of growth if glyphosate was not applied. The biological significance of those physiological indexes reflected on the plant dry mass accumulation that reduced by 61%. In this way, the relative tolerance to the herbicide of the clones GG100, C219, and I224 was of 1.1, 2.1, and 2.8 times higher than the clone I144 (with lowest GR50), respectively. Thus, the sequence of relative tolerance to glyphosate was I224 > C219 > GG100 ≥ I144.

Negative effects of simulated glyphosate drift on the eucalypt plant growth were also verified by other authors. Tuffi Santos et al. (2005), studying the dose-response of the eucalypt clone 15-CENIBRA (*E. urophylla* x *E. grandis*) to glyphosate, observed that the plant height and dry mass varied with the glyphosate subdoses, however it is not verified for the stem diameter. They also observed that plants exposed to glyphosate at 345.6 g a e ha^{-1} showed smaller plant height and dry mass values than plants exposed to lower glyphosate doses. Salgado et al. (2011) verified that glyphosate application at a low concentration (3%, v/v) was sufficient to reduce the leaf area exponentially of plantlet of *E. grandis* x *E. urophylla*. In addition, as the glyphosate concentration increased, the reduction was stronger. Deleterious effects of subdoses of glyphosate were also verified on other species or/and eucalypt hybrids (SANTOS et al., 2009; PEREIRA et al., 2010; COSTA et al., 2012a)

Glyphosate kills plants by inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (EC 2.5.1.19) of the shikimate pathway (DUKE et al., 2003). Inhibition of this enzyme results in reductions in shikimate pathway products, such as the aromatic amino acids phenylalanine, tyrosine, and tryptophan that are needed for protein synthesis and products of the these amino acids such as lignin, alkaloids, flavonoids, and benzoic acids necessary for cell wall development, defense against pathogens, and many other processes. Inhibition of EPSPS leads to high levels of shikimate accumulation due in part to unregulated flow of carbon into the shikimate pathway. This drains carbon from other pathways, leading to metabolic dysfunction. Glyphosate preferentially translocates to metabolic sinks, such as meristems and expanding cells, where it slows or stops plant growth.

There are many indirect effects of glyphosate, such as decreased levels of the activity of the enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) (AHSAN et al., 2008) and disorganization of the photosynthetic apparatus (MARÍA et al., 2005). Other general consequences of the exposure to glyphosate are chlorosis and plant growth reduction, mainly in metabolically active tissues, such as immature leaves, sprouts, floral buds, and root tips. At high glyphosate doses, these symptoms are followed by plant death.

Moreover, hormetic effect of low doses of glyphosate was observed in young plants of eucalypt, pine (*Pinus caribea*) (VELINI et al., 2008), and coffee (*Coffea arabica*) (CARVALHO; ALVES, 2012; CARVALHO et al., 2012a, 2013a). On the other hand, Tuffi Santos et al. (2006) and França et al. (2010) found no hormesis with glyphosate on coffee and eucalypt plants, respectively.

However, the hormetic effect of low doses of glyphosate is dependent on the plant species (TUFFI SANTOS et al., 2006), the growing conditions (BELZ; CEDERGREEN, 2010), the plant growth stage at the moment of the exposure to the herbicide (VELINI et al., 2008; CARVALHO et al., 2013a), and the end point measured and the time of its measurement after treatment (BELZ et al., 2011). Thus, with different experimental parameters, we may have observed hermetic effects.

Our results demonstrate a different susceptibility among the four clones of eucalypt to glyphosate. In addition, our data indicate that a glyphosate drift or even an accidental application of glyphosate directly to the shoot of all clones can reduce the initial plant growth, mainly of the clones GG100 and I144. Thus, a special care must be employed in using glyphosate for weed management in eucalypt plantations. Growers should be alert to the correct use and maintenance of the equipment and machinery, the choice of specific nozzles, the calibration of sprayers, the application under adequate environmental conditions, and so on.

Differences in the response of plants to the exposure to glyphosate can be derived from differences in spray retention (NORSWORTHY et al., 2001; MICHITTE et al., 2007) or/and drop contact angle (CHACHALIS et al., 2001; NORSWORTHY et al., 2001; MICHITTE et al., 2007) in the leaves, composition of the leaf epicuticular wax (MICHITTE et al., 2004; NANDULA et al., 2008; GUIMARÃES et al., 2009; HATTERMAN-VALENTI et al., 2011), herbicide absorption or/and translocation (DINELLI et al., 2008; GUIMARÃES et al., 2009; GE et al., 2010; CARVALHO et al., 2012b), and herbicide degradation (ROJANO-DELGADO et al., 2010, 2012; CARVALHO et al., 2012b, 2013b). Thus, any difference in the herbicide absorption, translocation, or/and metabolism can influence the plant response to glyphosate, culminating in a higher or lower tolerance to this herbicide.

Finally, under situations of potential risk of spray drift, such as herbicide applications in areas of rugged relief, upon poorly prepared soils (with many clods or soil unevenness that provide inadequate movement of the spray bar), in regions of occurrence of relatively strong winds, even considering the technology application is appropriate, there may be damage to eucalypt plant grow thif chosen a clone highly susceptible to glyphosate. Therefore, the clones GG100 and I144 should not be chosen (to be highly susceptible to glyphosate), giving preference to clones tolerant to glyphosate, such asI224 and C219.

5. CONCLUSION

The eucalypt clones respond differently to glyphosate exposure, so that among I224, C219, GG100, and I144, the susceptibility to the herbicide is increasing. Choosing a less susceptible clone, such as the I224, can be a guarantee of reducing risk of significant glyphosate intoxication that could be affecting the growth and yield of the crop of interest.

6. ACKNOWLEDGMENTS

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