## **GLYPHOSATE DRIFT IN EUCALYPTUS PLANTS**<sup>1</sup>

## Deriva de Glyphosate em Plantas de Eucalipto

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ABSTRACT - With the present study we aim to assess the damage caused to Eucalyptus plants exposed to glyphosate drift in different canopy portions. The drift simulation was carried out through application of 1,080 g ha<sup>-1</sup> of glyphosate in five canopy portions (0, 25, 50, 75 and 100% of the low branches), in four areas of cultivation. Areas I and II, plants with 0.91 and 2.98 m, and height of canopy drift exposition of 0.30 and 1.0 m, respectively. In areas III and IV both cultivations were 8.15 m high, varying the height of drift exposition between 2.0 and 2.5 m, respectively. At 30 and 480 days after application (DAA), the survival rate was assessed, and at 300 and 480 DAA diameter at breast height (DBH), height, volume and their respective increment were determined. The medium annual increment (MAI) was determined at 480 DAA. Area I, in which the plants were 0.91 m high, we observed that treatment with 100% of the low branches exposed to drift led to stand reduction of the plants around 18.75 and 38.19% at 30 and 480 DAA, respectively. Areas I and II showed reduction in plant growth in height and DBH, wood volume and MAI, to the extent that there was an increase in the portion of canopy exposed to glyphosate drift. However, in areas III and IV, in which 8.15 m height plants were found, no changes were verified for the evaluated characteristics, regardless of the portion of canopy exposed to glyphosate drift.

Keywords: wood production, herbicide, Eucalyptus spp.

RESUMO - Objetivou-se com o presente estudo avaliar os danos causados em plantas de eucalipto expostas à deriva de glyphosate em diferentes porções da copa. A simulação de deriva ocorreu por meio da aplicação de 1.080 g ha<sup>1</sup> de glyphosate em cinco porções da copa (0, 25, 50, 75 e 100%)dos ramos basais), em quatro áreas de cultivo: áreas I e II, plantas com 0,91 e 2,98 m e altura da copa exposta à deriva de 0,30 e 1,0 m, respectivamente; e nas áreas III e IV ambos os cultivos encontravam-se com 8,15 m de altura, variando a altura de exposição à deriva de 2,0 e 2,5 m, respectivamente. Aos 30 e 480 dias após a aplicação (DAA), foi avaliada a taxa de sobrevivência, e aos 300 e 480 DAA, determinou-se o diâmetro à altura do peito (DAP), altura das plantas, volume de madeira e os seus respectivos incrementos. Já o incremento médio anual (IMA) foi determinado aos 480 DAA. Na área I, em que as plantas se encontravam com 0,91 m de altura, observou-se, no tratamento com 100% dos ramos basais expostos à deriva, redução no estande de plantas na ordem de 18,75 e 38,19% aos 30 e 480 DAA, respectivamente. Nas áreas I e II constataram-se reduções no crescimento de planta em altura e DAP, no volume de madeira e no IMA, à medida que houve aumento da porção da copa exposta à deriva de glyphosate. Todavia, nas áreas III e IV, em que as plantas se encontravam com 8,15 m de altura, não se verificou alterações nas características avaliadas, independentemente da porção da copa exposta à deriva do glyphosate.

Palavras-chave: produção de madeira, herbicida, Eucalyptus spp.

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<sup>&</sup>lt;sup>1</sup> Recebido para publicação em 22.1.2015 e aprovado em 12.6.2015.

### INTRODUCTION

Weed management in eucalyptus homogeneous plantations is essential for the establishment and development of crops, especially in the first two years – this period of increased susceptibility to interference caused by different species living in the area.

Among the herbicides registered for eucalyptus, glyphosate, inhibitor of the enzyme EPSPs (5-enolpyruvylshikimate-3phosphate synthase), is the most widely used due to the broad spectrum of action and low environmental risk (Malik et al. 1989). However, the recurring use of glyphosate in weed management along the eucalyptus development cycle can become harmful to the crop because of accidental drift by manual and/or mechanized applications (Tuffi Santos et al. 2011). Studies report that glyphosate, when in contact with eucalyptus leaves, has caused considerable losses in wood increment and failures due to the death of least developed plants, reducing the stand (Tuffi Santos et al. 2005; 2007b).

Although there are studies evaluating the effects caused by the drift of eucalyptus saplings under controlled conditions (Tuffi Santos et al. 2005, 2007a, 2008; Veline et al. 2008), little is known about the effects thereof in more developed field crops in different stages of growth.

Given the above, the objective was to evaluate the intensity of the damage caused by the simulated glyphosate drift on eucalyptus plants according to the size and contact areas in the lower third of the eucalyptus canopy.

## MATERIALS AND METHODS

The experiment was carried out from May 2012 to September 2013 in areas of homogeneous eucalyptus plantations in the municipality of Santana do Paraíso, MG, with latitude 19°21'49'' S and longitude 42°34'07'' W and altitude 240 m. According to Köppen, the regional climate is classified as Aw, wet tropical type, with reduced rainfall in winter, showing monthly precipitation and average temperature of 106.50 mm and 22.8 °C, respectively. Planting was carried out in previously prepared and fertilized furrows, using clonal seedlings from the hybrid *Eucalyptus grandis* Hill ex Maden versus *Eucalyptus urophylla* Blade, clone CBN010, following the spacing of  $3.0 \ge 2.5 \text{ m}$ .

The research was divided into four trials regarding four areas of forest plantations, with different plant heights and/or canopy height subjected to glyphosate drift. Areas I and II have presented 0.91 to 2.98 m high plants, and the crown height exposed to drift of 0.30 and 1.0 m, respectively. As for the more developed fields, the eucalyptus plants were 8.15 m high, and the height submitted to drifting was 2.0 m (area III) and 2.5 m (area IV). In all tests, the experimental randomized block design with four replications and five treatments was used, which consisted of simulated drift at 25, 50, 75 and 100% of the basal area of eucalyptus in addition to control without herbicide. The experimental plots consisted of eight planting rows with eight eucalyptus plants, in a total of 64 plants per plot of 480 m<sup>2</sup>. As a useful area, the 36 central plants were considered, corresponding to an area of 270 m<sup>2</sup>.

Herbicide application was done with a manual backpack sprayer provided with a Yamaho spray nozzle (25), with a droplet size from coarse to very thick and pressure of 2 bar, at a dose of 1,080 g ha<sup>-1</sup>, with spray volume applied in 120 L ha<sup>-1</sup>.

At 30 and 480 days after the application (DAA) of glyphosate, eucalyptus plants dead due to herbicide action were observed, and then the percentage of survival was estimated.

In area I, since it presented smaller plants, DBH assessment at 0 DAA was not carried out. However, forest inventory and the average increase were performed at 0, 300 and 480 DAA in all trees of the plot floor area, with assessments of overall height with the aid of an inclinometer, respecting the maximum limit in the range of 130°, and diameter at breast height (DBH) assessments using a sliding T bevel (also known as a bevel gauge) graduated at 1.30 m above ground level. These variables were converted by means of calculations and turned into timber volume in bark using the following formula employed



by the forestry company where the work was conducted:

$$Vi = 0.01462 + 0.0000323 \times \left( \left( \frac{DBH}{10} \right)^2 \times \frac{Hgt}{100} \right)$$

where: Vi = individual volume (m<sup>3</sup>); DBH = diameter at breast height (cm); and Hgt = height (cm).

Also, the Average Annual Growth Rate (AAGR) was obtained at 480 DAA using the formula:

$$AAGR = \frac{VOB}{\frac{x}{365}}$$

where: AAGR = Average Annual Growth Rate (m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup>); VOB = timber volume over bark per hectare (m<sup>3</sup> ha<sup>-1</sup>); and x = total number of days for obtaining *VOB*.

For each experiment, the data on the survival rate were subjected to analysis of variance by F-test at 5% probability; in case of significance, regression equations were adjusted for the portions of the crown submitted to drift. In choosing the model, the biological explanation, the significance of the parameters and the regression coefficient were taken into account. As for the averages of the other variables, they were compared by Tukey test at 5% probability, as there was no regression models adjustment for these features.

#### **RESULTS AND DISCUSSION**

The survival rate evaluated at 30 and 480 days after application (DAA) was not influenced by glyphosate drift in different portions of the lower third of 2.98 m (area II) and 8.15 m (areas III and IV) high plants – data not shown. However, in area I, where eucalyptus was 0.91 m high, the survival rate decreased with increasing portion of the canopy reached by glyphosate, showing an average reduction of 18.75% and 38.19% of the stand when the plants had 100% basal part exposed to drift, at 30 and 480 DAA, respectively, compared to control (Figure 1).



*Figure 1* - Average survival rate of eucalyptus plants obtained in area I (0.91 m high plants and height exposed to drift of 0,30 m) at 30 (\_\_ → \_\_) and 480 (\_\_\_) days after application. Santana do Paraíso, MG.

Tuffi Santos et al. (2007a) have reported lower tolerance of eucalyptus saplings when subjected to glyphosate drift, taking these to death with the application of 345.6 g ha<sup>-1</sup>. However, in more developed plantations submitted to drift of 1,440 g ha<sup>-1</sup> of glyphosate, recovery of eucalyptus was observed when they had up to 21% of intoxication (Tuffi Santos et al. 2007b). Glyphosate influences the metabolic activity of the plants due to the blockage of aromatic amino acid synthesis and therefore the secondary components dependent of these (Buchanan et al. 2000) which are directly involved in the regulation of wood growth and quality (Veline et al. 2010). According to Veline et al. (2008) and Carvalho et al. (2012), plants tolerance to glyphosate is related to the concentration of herbicide intercepted and bigger development stage and therefore the reserve accumulation, as observed in areas II, III and IV whose plants were taller than 2.98 m (Table 1).

Increased crown portion reached by drift has provided significant reductions in plant growth in height, diameter at breast height (DBH) and volume of wood in smaller stands (areas I and II) at 300 DAA (Table 1). In area I, with plants treated with glyphosate drift in 100% of their basal branches, there was a reduced growth in height, DBH and volume around 33.19, 31.44 and 32.14%, respectively, compared to control. However, in area II (2.98 m high plants) lower level of damage was



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Table 1 - Eucalyptus plant growth grown in areas I, II, III and IV, submitted to glyphosate drift in different	crown proportions, at
30 days after application (DAA). Santana do Paraíso, MG	

Drift	DBH <sup>1/</sup>	Increase DBH	Height	Increase height	Volume	Increase volume
(%)	(cm)	(cm)	(m)	(m)	(m <sup>3</sup> per plant)	(m <sup>3</sup> per plant)
	Area I $-$ 0.91 m high plants and drift at 0.30 m in relation to ground level					
Control	7.146 a	_	7.846 a	6.471 a	0.028 a	-
25	5.927 b	_	6.346 b	5.306 b	0.023 b	-
50	5.235 bc	_	5.811 cb	4.906 cb	0.021 cb	-
75	5.051 c	_	5.392 cb	4.498 cb	0.020 cb	-
100	4.899 c	_	5.242 c	4.285 c	0.019 c	-
VC (%)	6.73	_	7.46	7.32	5.96	_
Area II – 2.98 m high plants and drift at 1.0 m in relation to ground level						
Control	8.409 a	5.042 a	10.508 a	6.558 a	0.039 a	0.023 a
25	7.973 b	4.903 ab	10.089 ab	6.357 a	0.036 ab	0.020 b
50	8.061 ab	4.796 ab	10.079 ab	6.189 a	0.036 ab	0.021 ab
75	8.098 ab	4.840 ab	9.991 ab	6.201 a	0.036 ab	0.021 ab
100	7.926 b	4.756 b	9.801 b	6.089 a	0.035 b	0.019 b
VC (%)	2.25	2.33	3.00	3.71	3.82	6.04
Area III – 8.15 m high plants and drift at 2.0 m in relation to ground level						
Control	10.180 ab	3.165 a	12.766 a	4.143 a	0.058 a	0.029 a
25	10.568 a	3.233 a	13.260 a	4.380 a	0.063 a	0.033 a
50	10.209 ab	3.059 a	12.472 a	3.709 a	0.057 a	0.028 a
75	10.168 ab	3.140 a	12.638 a	4.165 a	0.057 a	0.029 a
100	10.011 b	3.056 a	12.610 a	4.162 a	0.056 a	0.028 a
VC (%)	2.32	3.55	3.11	10.95	5.51	8.39
Area IV – 8.15 m high plants and drift at 2.5 m in relation to ground level						
Control	10.336 a	3.208 a	12.995 a	4.450 a	0.061 a	0.032 a
25	10.099 a	2.989 a	12.697 a	4.090 a	0.057 a	0.028 a
50	10.287 a	3.002 a	12.736 a	3.969 a	0.059 a	0.029 a
75	10.129 a	2.939 a	12.556 a	4.156 a	0.057 a	0.028 a
100	10.262 a	2.992 a	12.381 a	3.644 a	0.057 a	0.027 a
VC (%)	3.86	4.59	3.71	13.23	7.40	9.95

 $^{L'}$  Diameter at breast height (DBH). Means followed by at least one of the same letter in the column do not differ by Tukey test at 5% probability.

observed, with reductions around 6.72, 5.74 and 10.25% for the variables plant height, DBH and timber volume, respectively (Table 1).

In areas III and IV, the damage caused by glyphosate at 300 DAA was mitigated due to the greater eucalyptus stage of development at the time of application (Table 1). However, it can bee seen in area III that plants subject to drift in 25% of the canopy obtained gains of 5.27% in DBH compared to others exposed to 100%, both at a height of 2.0 m (Table 1). This was not observed in area IV, which was submitted to drift at 2.50 m from ground level (Table 1).

According to Taiz & Zeiger, (2006), plants in the early stages of development partition large parts of photoassimilates for the primary growth in order to promote competition for light at the expense of the accumulation of photoassimilates. Thus in saplings, as seen in areas I and II, it is possible to observe an increase in the photosynthetically active eucalyptus area (green leaves and branches), enhancing the damage by drift (Figure 1,



Table 1), since the green branches can absorb and translocate larger amounts of glyphosate to other parts of the plant, when compared to the older parts (Pereira et al. 2011; Costa et al. 2012).

As regards to homogeneous plantings, with the development of the crop, basal leaves and branches are subject to self-shading, which triggers their senescence process (Zanon et al. 2013). Also, leaves and branches already developed show poor anatomical and physiological plasticity when exposed to conditions of constraints in brightness, which promotes degradation of the photosynthetic apparatus and, consequently, remobilization of nutrients to the drains (Saur et al. 2000; Hay & Poter, 2006). Thus, with degrading chloroplasts, there is the ability to reduce the source capacity and therefore the translocation of photoassimilates (Hay & Poter, 2006) and glyphosate throughout the plant, thus mitigating the damage caused by the herbicide (Tables 1, 2 and 3).

*Table 2* - Eucalyptus plant growth grown in areas I, II, III and IV, submitted to glyphosate drift in different crown proportions, at 480 days after application (DAA). Santana do Paraíso, MG

Drift (%)	DBH <sup>1/</sup> (cm)	Increase DBH (cm)	Height (m)	Increase height (m)	Volume (m <sup>3</sup> per plant)	Increase volume (m <sup>3</sup> per plant)
Area I $-$ 0.91 m high plants and drift at 0.30 m in relation to ground level						
Control	9.705 a	2.560 b	12.989 a	5.144 a	0.055 a	0.027 a
25	9.195 a	3.269 a	11.799 b	5.453 a	0.049 b	0.026 a
50	8.506 b	3.271 a	11.164 bc	5.353 a	0.043 c	0.022 b
75	8.501 b	3.450 a	10.958 bc	5.567 a	0.042 c	0.023 b
100	8.428 b	3.529 a	10.754 c	5.512 a	0.041 c	0.022 b
VC (%)	2.64	7.29	3.74	5.59	4.76	5.83
Area II – 2.98 m high plants and drift at 1.0 m in relation to ground level						
Control	10.011 a	1.602 a	14.091 a	3.583 a	0.061 a	0.022 a
25	9.500 b	1.527 a	13.522 ab	3.433 a	0.055 ab	0.019 a
50	9.570 ab	1.509 a	13.589 ab	3.510 a	0.057 ab	0.020 a
75	9.682 ba	1.584 a	13.735 ab	3.744 a	0.057 ab	0.021 a
100	9.480 b	1.555 a	13.287 b	3.485 a	0.054 b	0.019 a
VC (%)	2.30	7.43	2.22	7.75	4.59	8.92
Area III – 8.15 m high plants and drift at 2.0 m in relation to ground level						
Control	11.474 ab	1.294 a	16.166 a	3.401 a	0.085 ab	0.027 a
25	11.839 a	1.271 a	16.581 a	3.320 a	0.090 a	0.027 a
50	11.467 ab	1.258 a	15.967 a	3.495 a	0.084 ab	0.027 a
75	11.438 ab	1.270 a	16.106 a	3.468 a	0.084 ab	0.026 a
100	11.238 b	1.226 a	15.966 a	3.356 a	0.081 b	0.025 a
VC (%)	1.98	6.17	2.12	8.46	4.20	5.63
Area IV – 8.15 m high plants and drift at 2.5 m in relation to ground level						
Control	11.718 a	1.383 a	15.739 a	2.744 a	0.086 a	0.026 a
25	11.426 a	1.327 a	15.495 a	2.798 a	0.082 a	0.024 a
50	11.614 a	1.326 a	15.376 a	2.640 a	0.083 a	0.024 a
75	11.378 a	1.249 a	15.615 a	3.059 a	0.082 a	0.024 a
100	11.516 a	1.254 a	15.430 a	3.048 a	0.082 a	0.025 a
VC (%)	3.50	5.85	3.40	19.27	7.81	13.40

<sup>1/</sup> Diameter at breast height (DBH). Means followed by at least one of the same letter in the column do not differ by Tukey test at 5% probability.



*Table 3* - Average Annual Growth Rate (AAGR) in eucalyptus cultivated in areas I (0.91 m high plants), II (2.98 m high plants), III and IV (both 8.15 m high) with different crown areas and plants height exposed to drift of 0.30, 1.0, 2.0 and 2.5 m, respectively. Santana do Paraíso, MG

Drift (%)	Ι	II	III	IV	
$(m^{-3} ha^{-1} year^{-1})$					
Control	51.792 a	58.047 a	87.447 ab	86.846 a	
25	36.875 b	52.959 ab	91.587 a	85.062 a	
50	33.674 b	52.373 b	84.887 b	84.328 a	
75	30.312 cb	54.701 ab	82.862 b	83.617 a	
100	24.216 c	50.372 b	84.886 b	82.165 a	
VC (%)	11.26	4.49	3.26	7.80	

Means followed by at least one of the same letter in the column do not differ by Tukey test at 5% probability.

In area I at 480 DAA, the increases in DBH and plant height, where the drift reached 100% of lower branches, were 27.45 and 6.68% higher than the control, respectively (Table 2). This is due to reduced stand at 38.12%, caused by the death of eucalyptus plants (Figure 1), and lower interspecific competition for light, water and nutrients, favoring the plants that survived. Tuffi Santos et al. (2007b) report on the importance of maintaining homogeneous plantations as a way to mitigate the damage caused by intraclonal competition of plants with growth inhibited by glyphosate. However, in areas II, III and IV this phenomenon was not observed (Table 2) due to a more uniform growth of the forest, since there was no effect of drift on the plants survival.

The largest single increase in plant height, DBH and volume due to the stand reduction (Table 2), as mentioned in area I, was not enough to offset the lower plant survival rate due to glyphosate drift on the Average Annual Growth Rate (AAGR) at 480 DAA, with higher loss rates observed as the surface area exposed to the herbicide (Table 3) increased, reaching AAGR loss around 53.24% when the plants had 100% of their area basal exposed to drift. In area II, with 2.98 m high plants, although there was no decrease in survival rate, it was also found in the AAGR due to glyphosate drift, with greater reduction rate as the exposed area was increased, as in area I, but with lower loss levels (Table 3).

In area IV, where the plants were 8.15 m high and exposure to drift was 2.50 m, there was no variation in AAGR (Table 3). On the

other hand, in area III there were higher rates of AAGR in the treatment with 25% of the crown, up to 2.0 meters high, exposed to drift, and in the control without herbicide application, compared to treatments with 50, 75 and 100% of the basal part of the crown submitted to drift.

The highest rates observed when plants had 25% of their basal area exposed to the herbicide are possibly due to the effect of hormesis, which may have favored the gain in DBH, wood volume (Table 2) and AAGR (Table 3). Tuffi Santos et al. (2007b) have observed that eucalyptus plants with 11 to 20% of intoxication caused by the glyphosate drift at 1,440 g ha-1 had an increase of 3.64% in volume when compared to the control without application. Veline et al. (2008) report that underdoses of glyphosate have favored growth of Eucalyptus grandis, Zea mays, Pinus caribea, Glycine max and Commelina benghalensis. For coffee crops, Carvalho et al. (2013), evaluating the application of glyphosate in doses of 500 g ha<sup>-1</sup>, at 45 days after transplanting, saw additive effects attributed to hormesis.

Thus, the factors related to the senescence process of the shaded leaves (Saur et al. 2000) and the degradation of the photosynthetic apparatus by glyphosate (Mcallister & Haderlie, 1985), associated with low area of exposure to drift, may have allowed the translocation of underdoses of glyphosate for the points of eucalyptus growth, triggering the process of hormesis (Veline et al. 2008; Carvalho et al. 2012, 2013; Belza & Duke, 2014). For Duke et al. (2006), hormesis influenced by glyphosate is due to the decrease



of the aromatic amino acids phenylalanine and tyrosine, which are precursors of lignin, reducing the stiffness of the cell wall, which, in turn, promotes cell elongation.

However, studies should be performed to evaluate the quality of wood from plants submitted to glyphosate drift, since this herbicide reduces the production of lignin, which, according to Gomide (2006), favors gains in pulp quality for cellulose, reducing expenses with reagents.

Given the above, it is concluded that eucalyptus trees in early growth stages are more sensitive to glyphosate drift, increasing the damage as the affected area is increased and can result in reduced survival rates. However, more developed plants, taller than 8.15 m, are tolerant to glyphosate drift in the lower third of the crown.

#### ACKNOWLEDGMENT

To Celulose Nipo-Brasileira (CENIBRA) for the financial and technical support in the performance of the experiments; to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Coordination of Improvement of Higher Education); and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; National Counsel of Technological and Scientific Development).

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