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EFFECTS OF GLYPHOSATE ON GROWTH, YIELD AND WOOD QUALITY OF *EUCALYPTUS UROGRANDIS*

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This paper aimed to understand glyphosate behavior on *Eucalyptus urograndis* plants with different ages in the field. Understanding these effects is essential to improve weed management and plant protection in eucalypt, since glyphosate spraying is the cheapest, fastest, most efficient and most used weed control method. Glyphosate was sprayed at 1, 3, 5, 7 and 9 months after planting using 0, 36, 72, 144, 288 and 432 g acid equivalent (ae) of glyphosate ha^{-1}. One month after planting, glyphosate doses up to 72 g ae ha^{-1} caused irreversible damage. Plants sprayed with glyphosate 5, 7 and 9 months after planting showed high recovery capacity. Accidental drift of glyphosate up to nine months after planting did not reduce the eucalypt growth parameters and wood volume. Only plants sprayed with higher doses (much larger than an accidental drift) showed reductions in wood volume. Thus, a dosage of almost twice more glyphosate is needed to cause reductions in the wood volume of older plants. However, trees sprayed at nine months after planting showed no reductions in productivity, even when sprayed with the higher dose of glyphosate. The spraying of glyphosate five months after planting did not affect the lignin content, holocellulose or basic density of the wood.

Keywords: Drift, herbicide, holocellulose, lignin, wood basic density, wood volume

INTRODUCTION

An efficient weed control is required for the establishment of successful forestry areas. Among different methods of eucalypt weed control, the chemical control is most common. Currently, glyphosate is the most used herbicide worldwide, providing control of many weed species (Steinmann et al. 2012, Bervald et al. 2010). Glyphosate accidental drift have promoted many discussions. Some studies indicate positive effects of subdoses or hormesis (Duke et al. 2006, Cedergreen & Olesen 2010, Pereira et al. 2013). Other studies indicate negative aspects related to direct or indirect effects of the shikimic acid pathway, which is inhibited by glyphosate, even at subdoses in some cases. Herbicides drift effects are directly linked to the species, plant age, specific weather conditions and the amount of herbicide absorbed.

Considering the above, glyphosate is directly involved with wood growth regulation. However, perennial plants such as eucalypt remain in the field for many years, and have a great recovery potential. Most studies have evaluated glyphosate effects for short periods, rarely exceeding few months, and usually spraying on the top of young seedlings which are notoriously susceptible to glyphosate drift. Glyphosate effects in plants with different ages in the field, assessed for months or years on its effect on wood productivity and quality, are scarce. However, such knowledge is essential for eucalypt which receives glyphosate as weed control at least once a year, and remaining in the field for long years with a harvest presenting 'good wood quality' to comply with the pulp and paper market requirements (i.e. basic density, wood volume, holocellulose and lignin contents). In this study, the hypothesis that eucalypt plants can have different effects according to plant age and glyphosate dose sprayed, was tested. The aim of the study was to understand the effects of glyphosate, sprayed on *Eucalyptus urograndis* under field conditions, and in plants with different ages as well as its long-term behavior on plant growth, yield and wood quality, five years after planting.

MATERIALS AND METHODS

Experimental site

The experiment was carried out from 2004 to 2009 in Luiz Antônio, São Paulo, Brazil, located at 21° 33' S, 47° 54' W, with an area of 582 m^2. According to Koppen Geiger climate classification, the local climate is classified as Cwa (humid subtropical climate). Highest quantity of rain is received between November and March, and the period between May and September is characterised as dry season. June, July and August were the driest months of the year. Eucalypt seedlings (*Eucalyptus grandis* x *E. urophylla*) were used. The experimental area was prepared for planting using a subsoiler (30 cm deep) and fertilised with reactive phosphate (300 kg ha^{-1}). In April 2004, planting was performed using 3 m spacing between rows and 2 m spacing between plants. Seedlings were selected using health, height (approximately 25 cm) and age (approximately 90 days old) criterias. Five days after planting (DAP) 90 g $plant^{-1}$ of 04-28-06 (N-P-K) + 0.3% Cu and 0.7% Zn were applied. At 90 DAP, 115 g $plant^{-1}$ 10-00-20 (N-P-K) + 0.3% B and 2.4% Mg were applied. At 12 and 18 months after planting (MAP), 100 kg ha^{-1} KCl + 1.5% B were applied. Up to 18 months, no additional fertilisation was performed.

Experimental design and spraying conditions

Experimental design was arranged in a strip-plot design with a number of application, i.e. glyphosate was sprayed 1, 3, 5, 7 and 9 MAP at doses 0, 36, 72, 144, 288 and 432 g ae ha^{-1} of Roundup (360 g L^{-1}). Two eucalypt rows represented a 90 m long strip, totalizing 90 plants (45 in each row). For evaluation purposes, only 74 central plants were considered. Among them, three uniform groups of trees were selected, constituting tree replicates. In each group/replicate, data was collected from five representative trees. Between each plot was a border (three eucalypt rows without glyphosate spraying) to avoid accidental drift to other plots, which received different glyphosate doses or different spraying times.

The spraying technique differed according to plant age and size. Till 5 months after planting, glyphosate spraying was performed using a backpack sprayer at constant pressure of 2 bar (CO_2), with spray nozzles XR 110 02 spaced at 0.5 m, and 200 L ha^{-1} spray volume. After 5 months old, due to plant height, it was necessary to reduce the spray volume by half (100 L ha^{-1}) and spraying was performed laterally on both plant sides. The same backpack sprayer was used, but with spray nozzles XR 110 01. Plant height, stem and leaf dry mass were determined in 5 plants before each glyphosate spraying. Plant dry mass was obtained by drying the material in an oven with forced circulation at ±70 °C for 72 hours.

Parameters evaluated

In the first 4 seasons (glyphosate sprayed at 1, 3, 5 and 7 MAP) height, stem diameter and leaf dry mass were evaluated at 60 and 120 days after glyphosate spraying (DAS), except in season 4 where evaluations were performed at 60 DAS due to difficulty in transporting and storing eucalypt trees that had a high green mass. For these same reason, the assessments were not performed in season 5 (glyphosate sprayed 9 MAP). At 10, 13, 26, 48 and 62 MAP, the height and diameter at breast height (DAB) were evaluated. With these data, wood volume was calculated:

$$V\ (m^3\ ha^{-1}) = \{[0{,}00078 \times (DBH)^2] \times H\} \times PP$$

where, DBH = diameter at breast height (cm) measured at 1.30 m from soil, H = height (m) and PP = plant population per hectare. Total extractives (%) according to standard TAPPI T204 cm-97 (TAPPI 1998b), total lignin content (%) according to standard TAPPI T222 cm-98 (TAPPI 1998a), holocellulose (%) obtained by subtracting lignin content of initial sample weight and wood basic density (g cm^{-3}) according to standard ABNT NBR 11941-02 (ABNT 2003) were analysed in plants where glyphosate was sprayed 5 MAP (season 3). As a control, six samples were taken from plants free of glyphosate spraying and the same contents described above were obtained.

Statistical analysis

Wood volume data was subjected to a Boltzmann sigmoidal regression analysis ($y = [(A1-A2)/1+e^{(x-x0)}]$), using the MicroCal Origin v. 6.0 software, considering a model with time and dose effects. Total extractives, total lignin content, holocellulose and basic density of wood means were compared using the mean standard error.

RESULTS

Season 1—Glyphosate spraying one month after planting

At two months after spraying (MAS) of season 1, doses above 36 g ae ha^{-1} were sufficient to cause a small decrease in stem and leaf dry mass. However, visual damages were only observed for doses above 72 g ae ha^{-1} (Figure 1) (Table 1). After glyphosate spraying at 288 g ae ha^{-1}, plant growth was nearly stagnant until 4 MAS. At dose 432 g ae ha^{-1} (higher dose) there was drying and plant death. At 4 MAS, plant that received 36 g ae ha^{-1} exhibited normal growth, and it was no longer possible to discern this treatment from control when analysing stem and leaf dry mass (Figure 1). At 9, 12 and 25 MAS no effects were observed at 36, 72 and 144 g ae ha^{-1} in wood volume. The result showed the capacity of recovery of eucalypt plants. However doses above 144 g ae ha^{-1} of glyphosate caused reductions in wood volume (Figure 2).

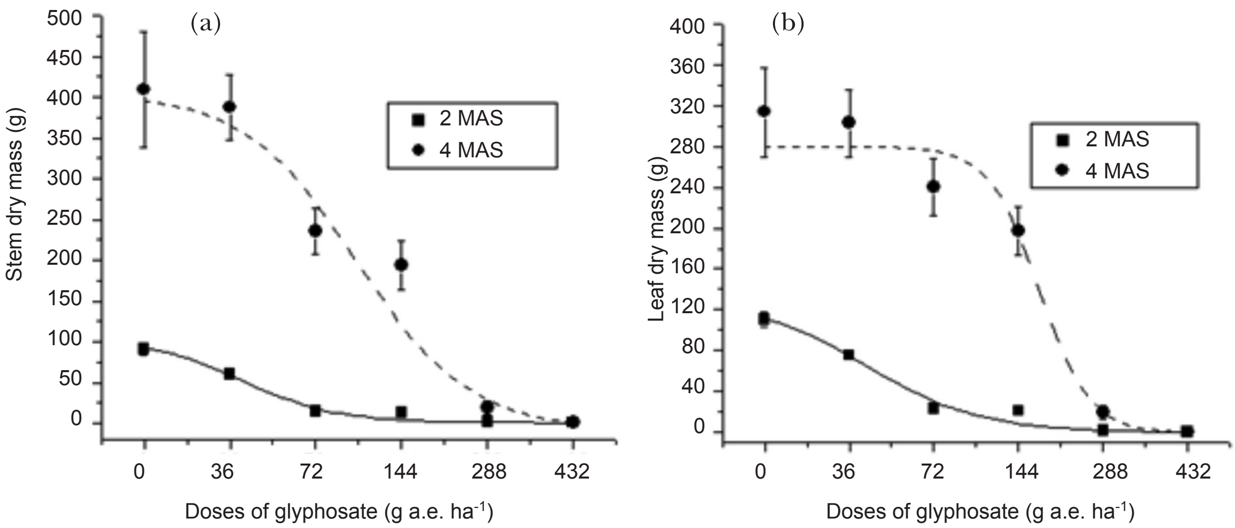

Figure 1 Stem (a) and leaf (b) dry masses of eucalypt subjected to glyphosate spraying one month after planting (season 1) at 2 and 4 months after spraying; the equations were adjusted using a Boltzmann sigmoidal regression analysis $y = [(A1\text{-}A2)/1+e^{(x\text{-}x0)}]$ considering the model with time and dose effects

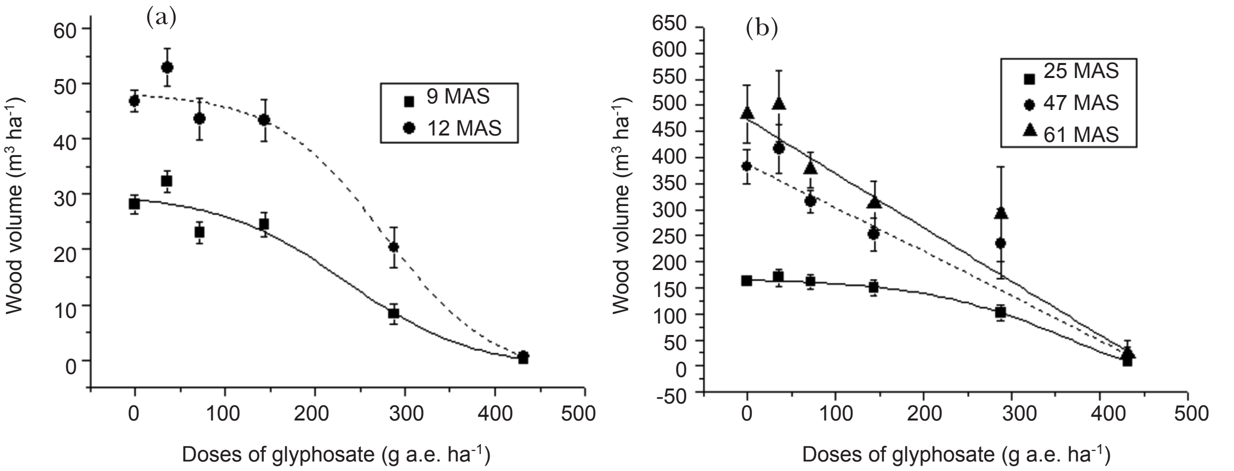

Figure 2 Wood volume (m^3 ha^{-1}) of eucalypt subjected to glyphosate spraying one month after planting (season 1) at 9, 12 (a), 25, 47 and 61 (b) months after spraying

Table 1 Equation parameters of Boltzmann sigmoidal regression analysis ($y = [(A1-A2)/1+e^{(x-x0)}]$) considering the model with time and dose effects

Season	Characteristic	Time	Equation parameters				
			R^2	A1	A2	x0	dx
1	Stem dry mass	2 MAS	0.98	104.95	104.95	2.13	0.54
		4 MAS	0.96	405.14	-8.15	3.47	0.65
	Leaf dry mass	2 MAS	0.98	131.72	-0.24	2.18	0.68
		4 MAS	0.99	279.8	-0.61	4.24	0.29
	Wood volume	9 MAS	0.99	30.15	-1.74	234.43	71.18
		12 MAS	0.99	48.41	3.00	276.77	60.47
		25 MAS	0.99	167.4	-71.76	371.06	85.54
		47 MAS	0.99	2773.64	-1516.19	-271.7	1208.95
		61 MAS	0.97	14032.04	-3380.34	-3476.67	2765.27
2	Stem dry mass	2 MAS	0.99	331.00	85.37	4.99	0.31
		4 MAS	0.99	890.36	274.47	4.22	0.55
	Leaf dry mass	2 MAS	0.95	253.93	68.75	5.04	0.37
		4 MAS	0.97	561.19	67.66	5.12	1.31
	Wood volume	7 MAS	0.96	31.35	10.64	260.12	37.98
		10 MAS	0.90	49.70	22.12	286.81	20.43
		23 MAS	0.99	165.73	5.37	473.45	102.63
		45 MAS	0.91	408.78	-1151.38	825.10	215.23
		59 MAS	0.98	510.74	-3502.12	900.71	182.05
3	Stem dry mass	2 MAS	0.91	521.86	984.16	4.19	-0.25
		4 MAS	0.54	3159.30	1991.40	5.29	0.48
	Leaf dry mass	2 MAS	0.85	687.40	374.58	3.04	0.38
		4 MAS	0.32	1401.40	644.31	6.47	0.59
	Wood volume	5 MAS	0.64	3.10	21.54	295.85	9.03
		8 MAS	-0.01	42.06	46.09	310.88	-2.67
		21 MAS	0.38	167.41	122.84	296.47	11.5
		43 MAS	-0.01	372.76	373.57	-6.66	3.15
		57 MAS	0.98	483.16	391.40	252.10	25.34
4	Stem dry mass	2 MAS	0.81	2930.30	1892.20	3.97	0.05
	Leaf dry mass	2 MAS	0.04	140.50	1298.70	5.00	0.07
	Wood volume	3 MAS	0.92	140.50	-216.01	1479.80	1849.67
		6 MAS	0.99	47.53	-14.36	461.37	102.64
		19 MAS	0.97	178.92	-1160.47	1366.67	306.60
		41 MAS	0.92	393.34	185.71	416.04	14.19
		55 MAS	0.90	490.11	231.24	417.10	12.95
5	Wood volume	1 MAS	0.00	28.72	28.74	153.95	-143.31
		4 MAS	0.03	46.85	45.97	35.23	0.37
		17 MAS	0.71	166.53	153.85	3.20	2.95
		39 MAS	0.92	363.70	523.91	275.03	8.54
		53 MAS	0.81	483.24	551.09	410.67	7.41

MAS = months after spraying

Season 2 – Glyphosate spraying three months after planting

In season 2, a decrease in plant growth with increasing glyphosate doses were observed. However, to promote growth reductions 2 MAS in season 2, doses greater than 144 g ae ha^{-1} were necessary, indicating a lower sensitivity of plants to glyphosate when compared to season 1 (Figure 3).

Between seasons 1 and 2, there was an increase of 30.8 times in stem dry mass and 23.7 times in the leaf dry mass. Thus, the 'dilution effect' should be considered, once the leaf and stem dry mass showed a large increase. In season 2, 432 g ae ha^{-1} caused symptoms of phytointoxication. However, the dose was not enough to cause plant death (which was observed in season 1). Even with high toxicity at 4 MAS, stem and leaf dry mass increased by 632.9 and 270.9%, respectively (control plant growth increased 2165.5 and 741.6%). For productivity at 7 MAS (Figure 4), doses above 220 g ae ha^{-1} were necessary to initiate wood volume reduction. Thus, almost twice more glyphosate was necessary to begin reduction in the wood volume of eucalypt at 10 MAS (season 2) (Figure 4a).

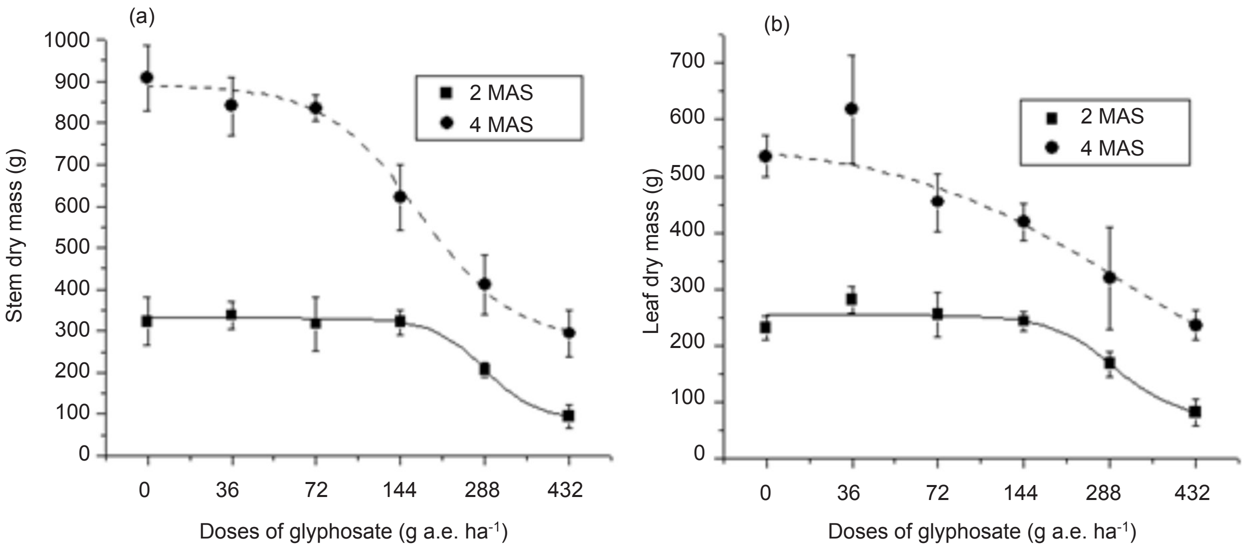

Figure 3 Stem (a) and leaf (b) dry masses of eucalypt subjected to glyphosate spraying two months after planting (season 2) at 2 and 4 months after spraying

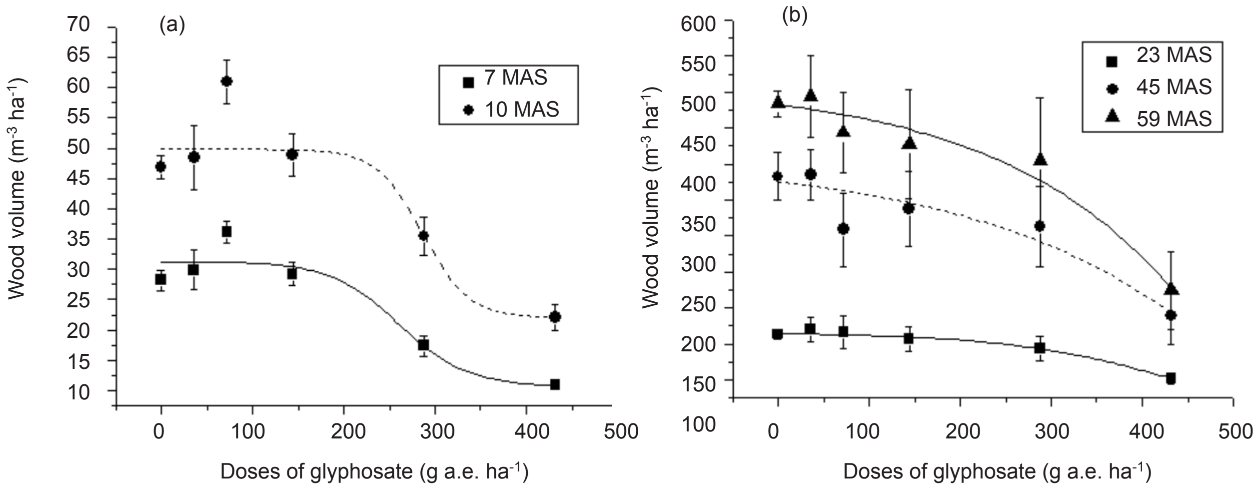

Figure 4 Wood volume (m^3 ha^{-1}) of eucalypt subjected to glyphosate spraying three months after planting (season 2) at 7, 10 (a), 23, 45 and 59 (b) months after spraying

From 23 to 59 MAS, only doses above 300 g ae ha^{-1} of glyphosate caused decrease in wood volume. It is well above the dosage that is considered accidental drift (10% of the recommended dose applied).

Season 3 – Glyphosate spraying five months after planting

Reductions caused by glyphosate in season 3 were less severe than seasons 1 and 2. The results were related to the 'dilution effect', within the dry and cold months in which glyphosate was applied. Once again, doses greater than 144 g ae ha^{-1} were required to decrease dry mass accumulation, 2 MAS. At 4 MAS, plants that received doses equal to or greater than 144 g ae glyphosate ha^{-1} showed recovery (Figure 5).

Results of wood volume indicated that 5 MAS doses above 250 g ae ha^{-1} were necessary to decrease the productivity. After 5 MAS, wood volume at the upper asymptote of sigmoidal regression (maximum yield) was 30 m^3 ha^{-1}, while at the lower asymptote (minimum yield) was 22 m^3 ha^{-1} (Figure 6). At 57 MAS, differences between highest and lowest production as a function of glyphosate doses was 163.8 m^3 ha^{-1}.

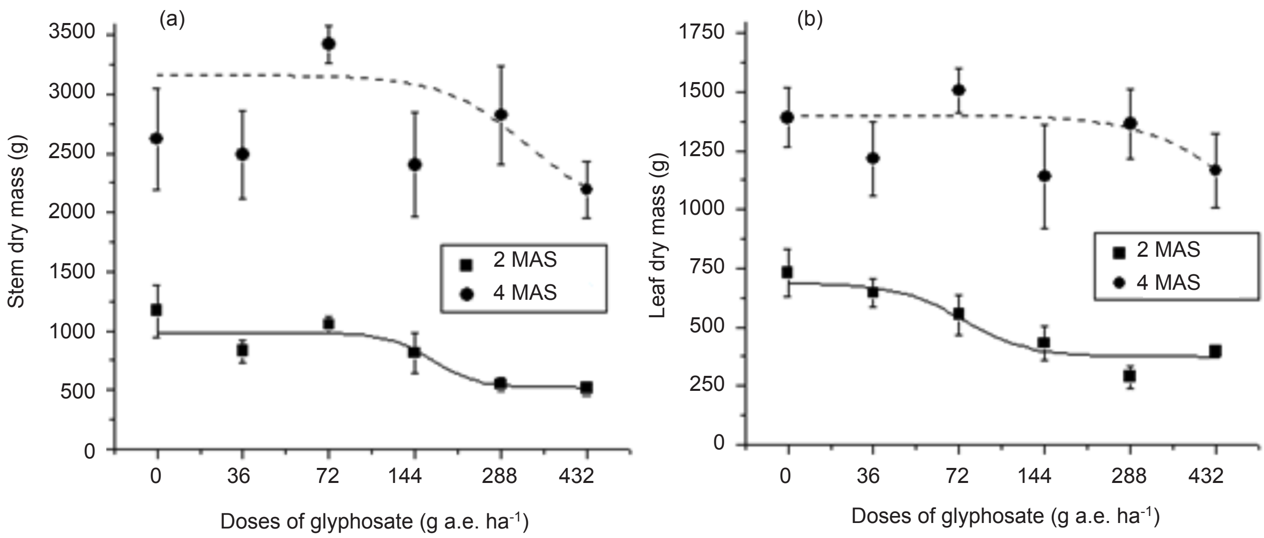

Figure 5 Stem (a) and leaf (b) dry masses of eucalypt subjected to glyphosate spraying five months after planting (season 3) at 2 and 4 months after spraying

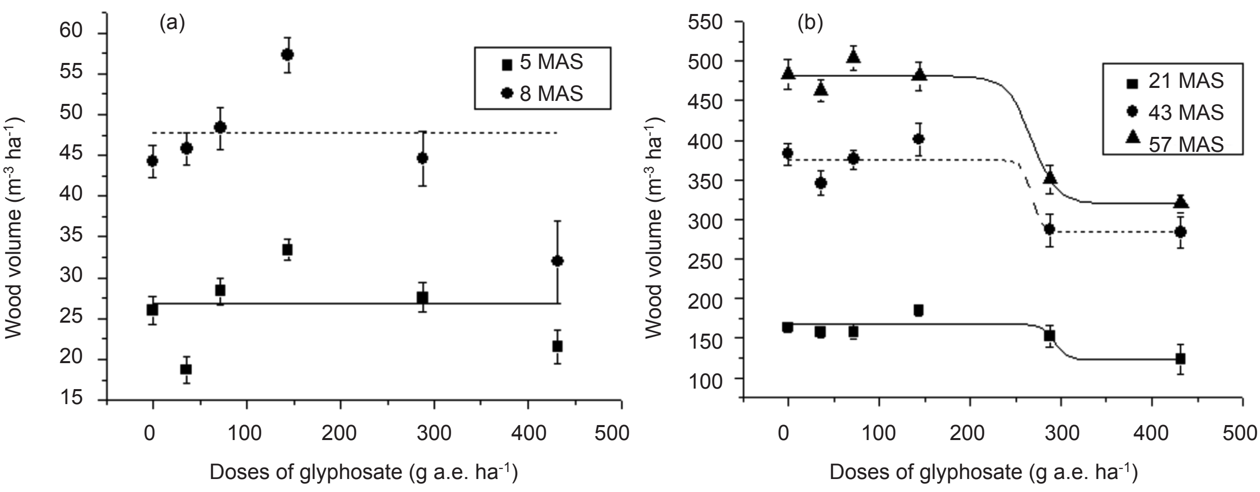

Figure 6 Wood volume (m^3 ha^{-1}) of eucalypt subjected to glyphosate spraying five months after planting (season 3) at 5, 8 (a), 21, 43 and 57 (b) months after spraying

The maximum yield was 483.2 m^3 ha^{-1} while the minimum yield was 319.4 m^3 ha^{-1}, indicating that even with higher doses of glyphosate eucalyptus plants could partially recover (Figure 6).

Season 4 – Glyphosate spraying seven months after planting

At 2 MAS, doses greater than 144 g ae ha^{-1} were sufficient to decrease stem and leaf dry masses (Figure 7). In season 4, doses from 144 g ae ha^{-1} were sufficient to decrease wood volume at 3 MAS. At 6 and 19 MAS, only doses greater than 200 g ae ha^{-1} decreased productivity. However, at 41 and 55 MAS, even doses greater than 350 g ae ha^{-1} maintained productivity levels, lower than expected (Figure 8).

Season 5 – glyphosate spraying nine months after planting

In contrast to previous seasons, in season 5 wood volume reductions were not observed (Figure 9). It was very likely that glyphosate dilution effect on eucalyptus plants was the main cause of the lack of response.

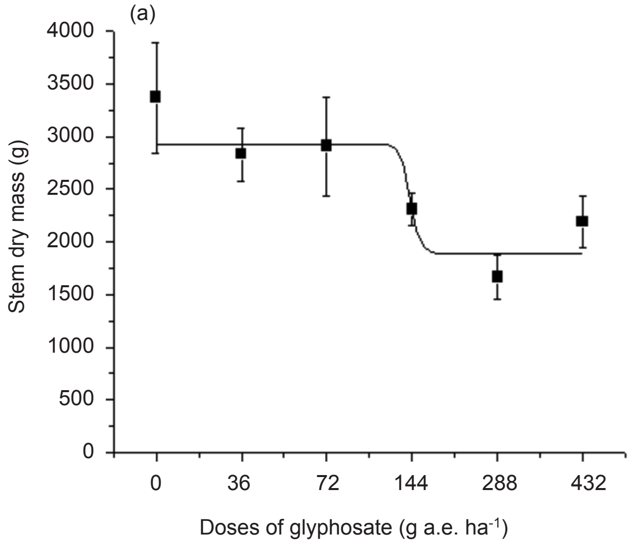

Figure 7 Stem (a) and leaf (b) dry masses of eucalypt subjected to glyphosate spraying seven months after planting (season 4) at 2 months after spraying

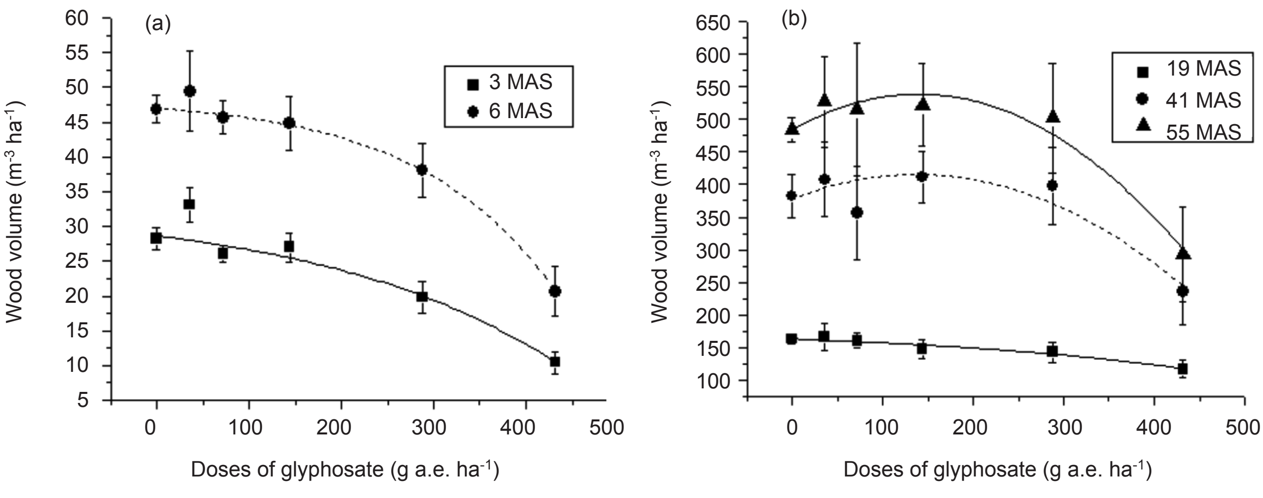

Figure 8 Wood volume (m^3 ha^{-1}) of eucalypt subjected to glyphosate spraying seven months after planting (season 4) at 3, 6 (a), 19, 41 and 55 (Bb) months after spraying

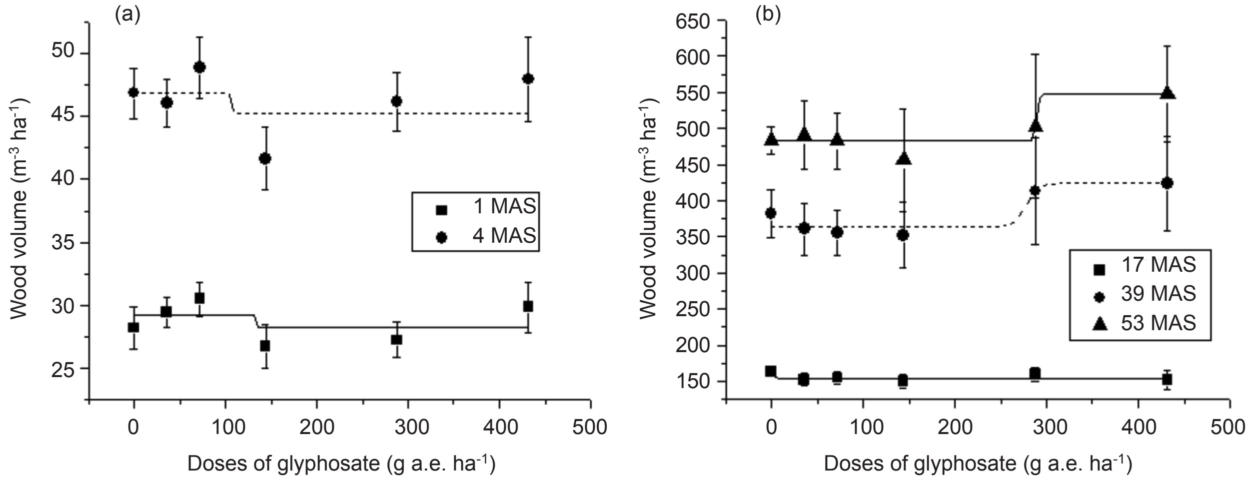

Figure 9 Wood volume (m^3 ha^{-1}) of eucalypt subjected to glyphosate spraying nine months after planting (season 5) at 1, 4 (a) 17, 39 and 53 (b) months after spraying

Total extractive content, total lignin content, holocellulose and wood basic density of eucalyptus submitted to glyphosate doses sprayed at 150 days after planting (season 3)

Representing less than 5% of total sample, the extractive content exhibited great variation (Figure 10a). In the free-spraying control, extractive content was 3.5% greater than the historical average values (2.4% in horizontal line). Plants sprayed with glyphosate presented values between 1.4 and 2.3%. Wood density (Figure 10b), total lignin content and holocellulose (Figure 11) did not change as a function of glyphosate spraying in season 3 (150 DAP). No significant variations were found to lignin, holocellulose, extractives and wood density due to glyphosate doses in season 3. The values obtained did not promote changes in chemical processes, to obtain pulp and cellulose.

DISCUSSION

Glyphosate effects in eucalypt seedlings (1 to 3 months after planting) are well known in literature and can compromise productivity and wood quality (Tuffi Santos et al. 2005). However, when glyphosate is accidentally sprayed in older plants (3 or more MAP) the effects of glyphosate were less severe. *Eucalyptus urophylla* plants exposed to glyphosate drift (1080 g ae ha^{-1}) in different canopy portions (0, 25, 50, 75 and 100% of low branches) in four areas, showed reduction in plant growth, height, medium annual increment and wood volume, especially in areas with shorter trees. But in areas with taller trees no changes were found (Santos Junior et al. 2015). Possibly, a large volume of leaves promoted a 'dilution effect'. In addition to a higher leaf area retaining the herbicide, other factors negatively influenced the glyphosate penetration. Herbicide foliar absorption is directly related to the structures found in leaves and the permeability of cuticle (Baker 1982). Leaf hydration is very important to glyphosate absorption, and hydrated cuticles are more permeable. In this experiment, some glyphosate sprayings were held in dry months, or after dry seasons, which possibly resulted in leaf morphological alterations, and consequently glyphosate penetration and action.

In young leaves, the metabolic activity and nutrient consumption are high, resulting in leaves more predisposed to input compounds. Thinner cuticle, smaller amount of waxes and cutin, and a larger amount of pectin, which is more hydrophilic, can also facilitate the absorption (Schreiber 2005). Despite plant growth throughout 150 DAP in season 3 and 210 DAP in season 4, plants exhibited increased sensitivity to glyphosate. This fact can be attributed to good humidity and temperature conditions which may have made easy glyphosate absorption and translocation. Due to the size of plants, glyphosate was sprayed in two different methods. This change may have increased glyphosate penetration into the plant and foliar retention. Volume spraying reduction may be favored to glyphosate absorption.

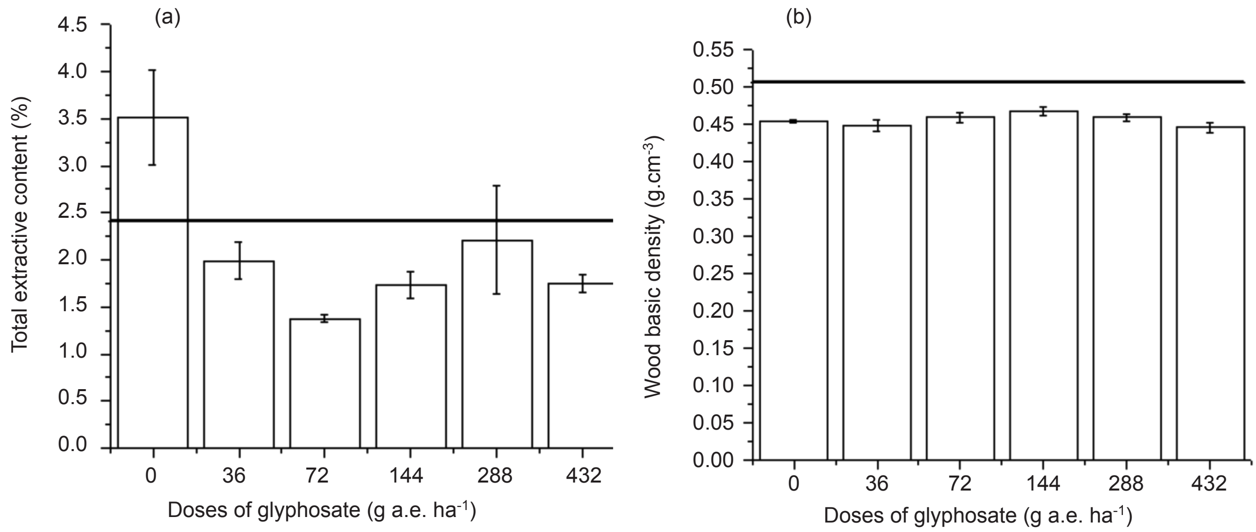

Figure 10 Total extractive content (a) and wood basic density (b) of eucalypt subjected to glyphosate five months after planting (season 3) and evaluated 68 months after planting

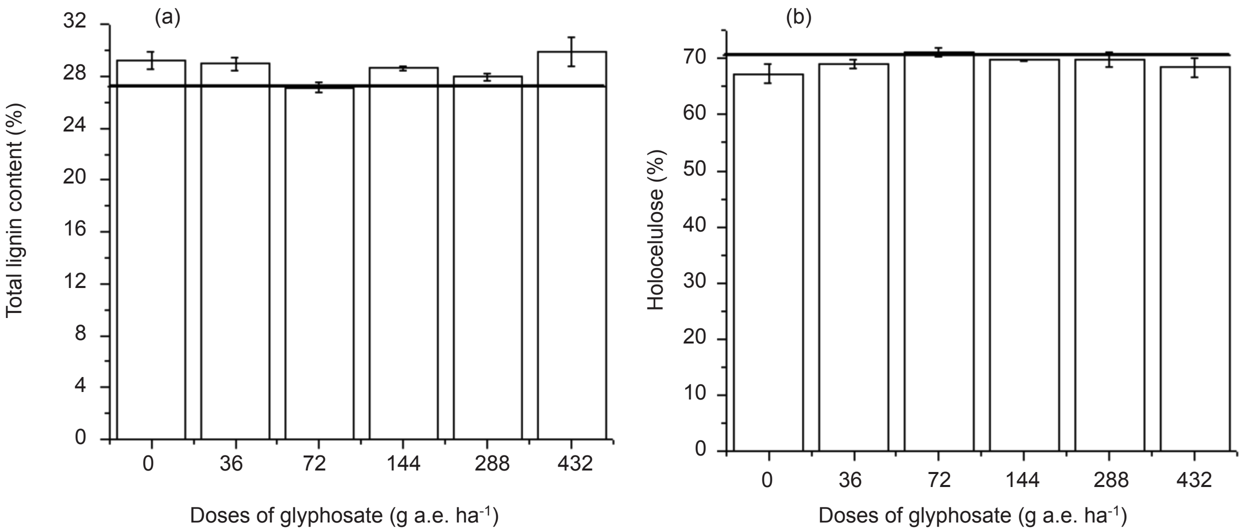

Figure 11 Total lignin content (a) and holocellulose (b) of eucalypt subjected to glyphosate five months after planting (season 3) and evaluated 68 months after planting

In general, plants sprayed with glyphosate 5, 7 and 9 MAP showed high recovery capacity. This information, first for eucalypt, was found in other perennials, such as citrus. Glyphosate effects at 0, 180, 360 and 720 g ae ha^{-1} in *Citrus limonia* seedlings under controlled conditions, showed that the metabolism of plants was affected until eight DAS and only transitory effects were observed (Gravena et al. 2009). From 6–12 months after treatment, plants were completely recovered. Due to the great capacity of recovery, only plants sprayed with higher doses (much larger than an accidental drift) showed reductions in wood volume.

For cellulose production, extractive compounds are undesirable because they reduce productivity. However, significant changes in extractive content, total lignin content and holocellulose were not found in plants sprayed 5 MAP. Lignin production is controlled by phenylalanine, thus, the pathway can be highly affected by glyphosate. Modified soybean plants treated with glyphosate have been shown to produce less lignin when compared to non-treated plants (Zobiole et al. 2010).

In this study, no differences were found. Information about glyphosate effects in lignin and holocellulose contents in plants are scarce. Studies of glyphosate effects on perennial crops, such as eucalypt, should not be restricted to short-term effects. Environment, weather, soil fertility, crop management, planting season, population density, species, clones and others can substantially modify the results over time.

CONCLUSIONS

Plants sprayed with glyphosate 5, 7 and 9 months after planting showed high recovery capacity. Accidental drift of glyphosate up to nine months after planting did not reduce the eucalypt growth parameters and wood volume. A dosage of almost twice more glyphosate is needed to cause reductions in the wood volume of older plants. However, trees sprayed at nine months after planting showed no reductions in productivity, even when sprayed with the higher dose of glyphosate. The spraying of glyphosate five months after planting did not affect the lignin content, holocellulose or basic density of the wood.

REFERENCES

ABNT (Associação Brasileira De Normas Técnicas). 2003. *NBR 11941: Madeira–Determinação da Densidade Básica.* ABNT, Rio de Janeiro.

Baker EA. 1982. Chemistry and morphology of plant epicuticular waxes. Pp 140–161 in Cutler, HA, Winnwe WE & Pell EJ (eds) *The Plant Cuticle.* Academic Press, London.

Bervald CMP, Mendes CR, Timm FC, Moraes DM, Barros Acsa & Peske ST. 2010. Desempenho fisiológico de sementes de soja de cultivares convencional e transgênica submetidas ao glifosato. *Revista Brasileira de Sementes* 32: 09–18.

Cedergreen N & Olesen CF. 2010. Can glyphosate stimulate photosynthesis? *Pesticide Biochemistry and Physiology* 96: 140–148.

Duke SO, Cedergreen N, Velini ED & Belz RG. 2006. Hormesis: is it an important factor in herbicide use and allelopathy? *Outlooks on Pest Management* 17: 29–33.

Gravena R, Victoria Filho R, Alves PLCA, Mazzafera P & Gravena AR. 2009. Low glyphosate rates do not affect *Citrus limonia* (L.) Osbeck seedlings. *Pest Management Science* 65: 420–425.

Pereira FCM, Nepomuceno MN, Pires RN, Parreira MC & Alves PLCA. 2013. Response of eucalyptus (*Eucalyptus urograndis*) plants at different doses of glyphosate. *Journal of Agricultural Science* 5: 66–74.

Santos Junior A, Tuffi Santos LD, Ferreira FA, Ferreira LR, Felix RC, Amaral GC & Cruz LR. 2015. Glyphosate drift in eucalyptus plants. *Planta Daninha* 33: 615–621.

Schreiber L. 2005. Polar paths of diffusion across plant cuticles: new evidence for an old hypothesis. *Annals of Botany* 95: 1069–1073.

Steinmann HH, Dickeduisberg M & Theuvsen L. 2012. Uses and benefits of glyphosate in German arable farming. *Crop Protection* 42: 164–169.

TAPPI (Technical Association of Pulpand Paper Industry). 1998a. *TAPPI Test Method T222 om-88: Acid - Insoluble Lignin in Wood and Pulp.* TAPPI Press, Atlanta.

TAPPI (Technical Association of Pulpand Paper Industry). 1998b. *TAPPI Test Method T204 cm-97: Solvent Extractives of Wood and Pulp.* TAPPI Press, Atlanta.

Tuffi Santos LD, Ferreira FA, Meira RMSA, Barros NF, Ferreira LR & Machado AFL. 2005. Crescimento e morfoanatomia foliar de eucalipto sob efeito de deriva do glyphosate. *Planta Daninha* 23: 133–142.

Zobiole LHS, De Oliveira RS, Kremer RJ, Constantin J, Bonato CM, Muniz AS. 2010. Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate. *Pesticide Biochemistry and Physiology* 97: 182–193.