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Article

Use of Fertiactyl Pos® for Protection of Eucalyptus Plants Subjected to Herbicide Drift

Uso do Fertiactyl Pós® na Proteção de Plantas de Eucalipto Submetido à Deriva de Herbicidas

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ABSTRACT - Herbicide drift, due to application failures, can cause intoxication in eucalyptus plants, compromising their growth. However, the addition of protective products such as Fertiactyl Pós® to the syrup has been shown to be efficient in reducing intoxication of eucalyptus plants exposed to herbicide drift. Given this, the objective of the present study was to evaluate the protective effect of Fertiactyl Pós® on the reduction of damage caused by the herbicides glyphosate, 2,4-D and the glyphosate + 2.4-D mixture in eucalyptus plants. The experiment was conducted in a 3 x 2 + 1 factorial scheme, corresponding to three herbicides: glyphosate (1,440 g ha^{-1}), 2,4-D (680 g ha^{-1}), glyphosate + 2.4- D (1,440 g ha^{-1} + 680 g ha^{-1}); two doses of Fertiactyl Pós® (0 and 3.0 L ha^{-1}) and one control without application. Prior to application, the top of the eucalyptus was covered with polyethylene bags to ensure the syrup only reached the lower third of the canopy. The addition of Fertiactyl Pós® to the syrup with glyphosate and 2.4 D herbicides reduced the intoxication of eucalyptus plants by 21.9 and 15.2%, respectively, at 40 days after application (DAA) when compared with the application of the respective herbicides without the addition of Fertiactyl Pós®. The glyphosate + 2.4-D mixture drift intensified the intoxication of the eucalyptus plants in comparison with the application of the respective herbicides in isolation. Besides, the addition of Fertiactyl Pós® to the glyphosate + 2,4-D mixture did not prevent plant intoxication.

Keywords: glyphosate, 2.4-D, plant detoxification, herbicide mixture.

RESUMO - A deriva de herbicida, por falhas de aplicação, pode causar intoxicação em plantas de eucalipto, comprometendo o crescimento destas. Entretanto, a adição de produtos protetores como Fertiactyl Pós® à calda tem se mostrado eficiente na redução da intoxicação de plantas de eucalipto expostas à deriva de herbicidas. Em face do exposto, objetivou-se neste trabalho avaliar o efeito protetor do Fertiactyl Pós® sobre a redução de danos causados pela deriva dos herbicidas glyphosate, 2,4-D e a mistura glyphosate + 2,4-D em plantas de eucalipto. O experimento foi conduzido em esquema fatorial 3 x 2 + 1, correspondendo a três herbicidas: glyphosate (1.440 g ha^{-1}), 2,4-D (680 g ha^{-1}) e mistura em tanque de glyphosate + 2,4-D (1.440 g ha^{-1} + 680 g ha^{-1}); duas doses de Fertiactyl Pós® (0 e 3,0 L ha^{-1}); e uma testemunha sem aplicação. Antes da aplicação, a parte superior da copa do eucalipto foi protegida com sacos de polietileno, de modo a permitir que a calda atingisse apenas o terço inferior do dossel. A adição do Fertiactyl Pós® à calda com os herbicidas glyphosate e 2,4-D reduziu a intoxicação das plantas de eucalipto em 21,9% e 15,2%, respectivamente, aos 40 dias após a aplicação (DAA), em relação à aplicação dos respectivos herbicidas sem adição de Fertiactyl Pós®. A deriva da mistura glyphosate + 2,4-D potencializou a

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intoxicação das plantas do eucalipto em relação à aplicação isolada dos respectivos herbicidas e não houve resposta da adição do Fertiactyl Pós® na redução da intoxicação das plantas quando se aplicaram os herbicidas em mistura.

Palavras-chave: glyphosate, 2,4-D, desintoxicação de plantas, mistura de herbicidas.

INTRODUCTION

Weed management in eucalyptus plantations is an important practice for crop establishment and growth. Among the weed control methods in the forest sector, glyphosate applications are the most used chemical method due to the large cultivated areas, low risk of environmental contamination and wide control over several weed species (Malik et al., 1989). However, herbicide drift due to application failures can result in plant poisoning (Tuffi Santos et al., 2006; Carvalho et al., 2014; Santos Junior et al., 2015), especially when drift occurs in the first two years after transplanting, a period of high glyphosate susceptibility for eucalyptus seedlings (Santos Junior et al., 2015).

The intensive use of glyphosate has selected for tolerant weeds in eucalyptus plantations (Tuffi Santos et al., 2013), which requires the adoption of alternative weed management techniques to reduce glyphosate selection pressure. The mixture of 2,4-D with glyphosate increases the weed control spectrum, reducing the selection pressures of glyphosate-tolerant annual eudicotyledonous species. However, tank mixing of these herbicides may increase the risk of plant poisoning by drift compared to glyphosate applications in isolation (Gandolfo et al., 2012).

Herbicide drift damage, especially that caused by glyphosate, in applications on eucalyptus and soybean crops has been shown to be less severe when mixed with Fertiactyl Pós® (Santos et al., 2015; Constantin et al., 2016; Machado et al., 2017). Fertiactyl Pós® contains humic and fulvic acids, as well as the GZA (glycine betaine and zeatin) complex. Humic and fulvic acids absorb herbicide molecules in their superstructures (Piccolo and Celano, 1994; Piccolo et al., 1996; Campos and Vieira, 2002; Mazzei et al., 2012), and the GZA complex helps recovery from damage caused by reactive oxygen species (Giri, 2011), and may also increase cell membrane stability and abiotic stress tolerance (Taiz and Zeiger, 2006).

The objective of this study was to evaluate the effect of Fertiactyl Pós® in mixture with glyphosate, 2,4-D and glyphosate + 2,4-D on the reduction of damage caused by the drift of these herbicides on eucalyptus plants.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse from July 2015 to January 2016, in Viçosa, MG (42º52’54" W, 20º45’10" S).

Standardized seedlings of *Eucalyptus grandis* Hill ex Maden x *E. urophylla* Blade clone (CNB-10) of 30 cm high and 3 month old were transplanted into 10 dm^{-3} pots filled with soil, whose chemical characteristics are described in Table 1. Soil pH was corrected with 0.3 g dm^{-3} limestone and fertilized with 1.68 g dm^{-3} triple superphosphate, 0.22 g dm^{-3} potassium chloride and 0.33 g dm^{-3} of ammonium sulfate. After transplantation, plants were irrigated when necessary to keep the soil close to field capacity.

The experiment was conducted in a randomized complete block design including three herbicide treatments (glyphosate at 1,440 g ha^{-1}, 2,4-D at 680 g ha^{-1} and glyphosate + 2,4-D tank mixture at 1,440 + 680 g ha^{-1}, respectively) and two doses of Fertiactyl Pós® (0 and 3.0 L ha^{-1}). A control without application was also realized and each combination had three replications, with one plant per pot being considered as experimental unit

Herbicide applications were performed 150 days after transplanting, when eucalyptus seedlings were ~110 cm high. The application was carried out using a precision CO_2-backpack sprayer equipped with two TT110.02 tips spaced 0.50 m apart, calibrated at 300 kPa to deliver

Table 1 - Soil chemical and particle size analysis

pH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	t	T	V	m	MO
(H_2O)	(mg dm^{-3})		(cmol$_c$ dm^{-3})							(%)		(dag kg^{-1})
4.4	1.1	23	0.1	0.1	1.7	7.43	0.26	1.96	7.69	3.0	87.0	2.75

Clay	Silt	Areia	Texture	Soil type
(%)				
57	21	22	Clay	Clayey

pH in water; P and K^+ – Mehlich 1 extractor; Ca^{2+}, Mg^{2+} and Al^{3+} – extractor: KCl – 1 mol L^{-1}; H + Al – extractor Calcium Acetate 0.5 mol L^{-1} pH 7.0; SB – sum of exchangeable bases; t – effective cation exchange capacity; T – cation exchange capacity at pH 7.0; V – base saturation index; m – aluminum saturation index; MO – organic matter.

150 L ha^{-1}. The upper 2/3 of the eucalyptus canopy was protected with polyethylene bags, allowing the herbicide syrup to reach only the lower third of the plants.

Eucalyptus plant poisoning evaluations were performed at 10, 20, 30 and 40 days after application (DAA), using the percentage scale proposed by the ALAM (1974), where 0 = absence of visible intoxication symptoms, and 100 = death of eucalyptus plants.

At 0, 10, 20, 30 and 40 DAA, plant height and stem diameter at 3.0 cm above ground level were measured in the eucalyptus plants. At 40 DAA, the shoots were cut and separated into stem and leaves. The leaf area of each plant was measured by the Li-Cor 3000 meter (Li-Cor Instruments). Plant sections were stored in paper bags, dried in a stove with forced air at 70 °C until reaching a constant mass, and then weighed.

The total dry matter of the aerial parts was obtained from the sum of the dry weight of leaves and stem, and the specific leaf area (AFE) was determined by the relation of leaf area of the plant with leaf dry matter (Hunt, 1990).

All variables were subjected to analysis of variance (ANOVA) at 5% probability. In cases of significance ($p > 0.05$), the percentage intoxication data, plant diameter and height were adjusted to regression equations, and the means were compared using the standard deviation of the mean. In choosing the model, the biological explanation, the significance of the parameters and the regression coefficients were considered.

The averages of the dry matter of leaves, stems and total, as well as leaf area and AFE, were compared to the control without application using the Dunnett test at 5% probability. Furthermore, the presence or absence of Fertiactyl Pós® for each herbicide was compared by the T test at 5% probability.

RESULTS AND DISCUSSION

Eucalyptus seedlings showed poisoning symptoms caused by glyphosate and 2,4 D drift, either in isolation or in mixture regardless of whether Fertiactyl Pós® was added or not, at the different evaluated time intervals (Figure 1). At 40 DAA, poisoning was 21.9 and 15.2% lower when glyphosate and 2,4-D, respectively, were applied alone or in mixture with Fertiactyl Pós® (Figures 1A and B). The application of glyphosate + 2,4-D increased the intoxication of eucalyptus plants compared to individual application of these herbicides, regardless of the addition of Fertiactyl Pós® (Figure 1C).

The lower intoxication associated with the slower response of eucalyptus to individually applied herbicides provided a longer period for Fertiactyl Póa® to play its protective role, in relation with the thank mixture application of these herbicides (Figure 1). Simulated drift on 50 cm height eucalyptus plants caused lower intoxication at 15 DAA when glyphosate (10.75%) and triclopyr (30%) were applied individually in comparison to the tank mixture of these herbicides (42.5%) (Tuffi Santos et al. 2006).

The interaction of glyphosate and 2,4-D with macromolecules of humic and fulvic acids by hydrogen bonding keeps these herbicides adsorbed on these superstructures (Piccolo and Celano, 1994; Piccolo et al., 1996; Campos and Vieira , 2002; Mazzei et al., 2012), which may reduce the

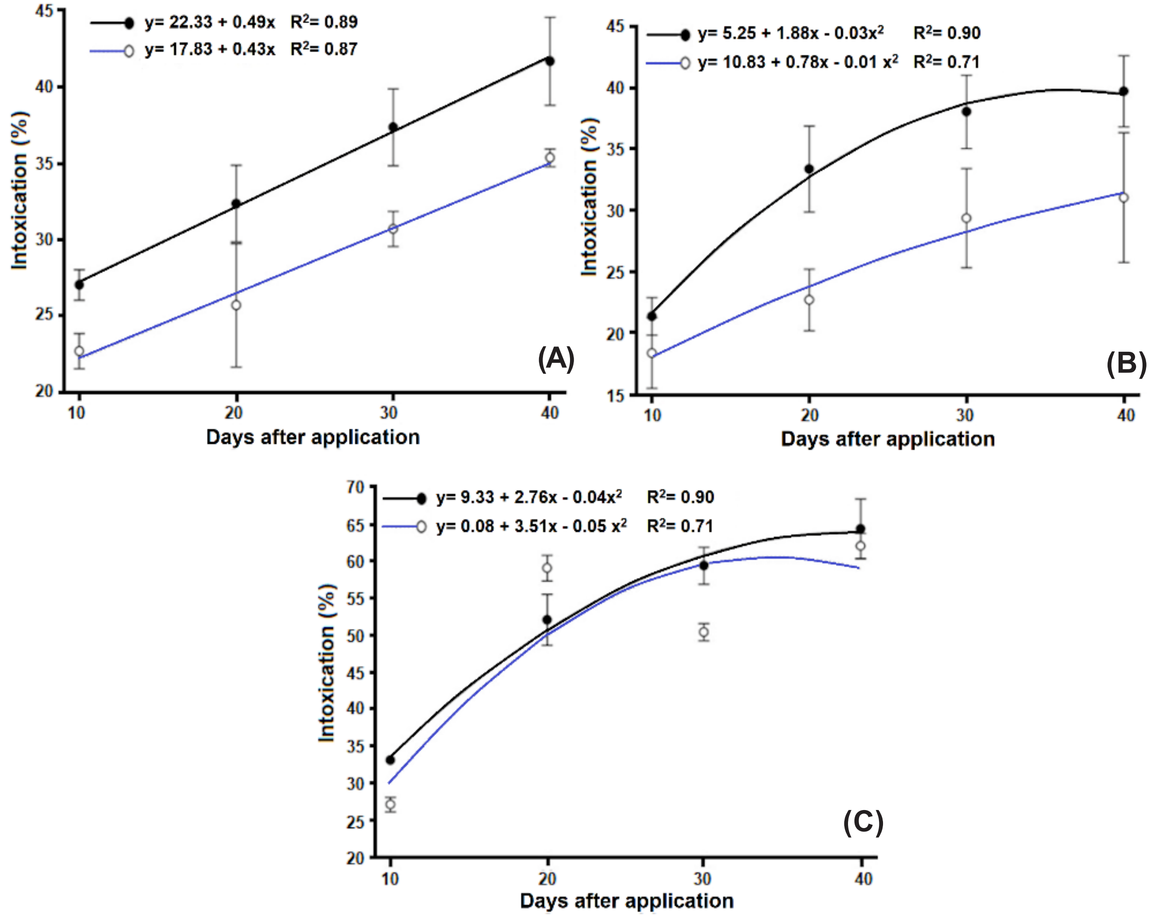

Figure 1 - Intoxication percentage of eucalyptus plants subjected to simulated drift of 2,4-D (A), glyphosate (B) and 2,4-D + glyphosate (C). with ( ) and without ( ) Fertiactyl Pós®.

toxic effect and explain the reduction in plant poisoning observed when Fertiactyl Pós® was added to the herbicide syrup (Figure 1A and B). Another aspect is related to the protective potential of these superstructures in mitigating damage caused by the accumulation of reactive oxygen species (ROS) due to abiotic stress (García et al., 2012). The presence of the GZA complex in the Fertiactyl Pós® composition may have contributed to the recovery of herbicide damage due to the protection of the photosynthetic apparatus, inhibition of leaf senescence, induction of genes involved in stress tolerance, reduction of ROS levels and cell membrane protection (Ashraf and Foolad, 2007; Chen and Murata, 2008; Wang et al., 2008; Giri, 2011).

In studies with increasing doses of glyphosate on eucalyptus plants of ~60 cm height, poisoning symptoms were observed from 1440 g ha^{-1} glyphosate mixed in the tank with 1.0 L ha^{-1} Fertiactyl Pós® (Machado et al. 2017). However, at doses higher than 1.0 L ha^{-1} Fertiactyl Pós®, glyphosate-related poisoning was not observed at the same dose, differing from the results obtained in this study, which may be related to the larger area exposed to drift (~110 cm tall plants). In glyphosate resistant soybean, a reduction in poisoning and grain yield gain were observed in plants submitted to increasing doses of Fertiactyl Pós® mixed with glyphosate + lactofen (Santos et al., 2015). The application of glyphosate, 2,4-D and glyphosate + 2,4-D provided a small increase in plant height when compared to the control without application, regardless of the addition of Fertiactyl Pós® (Figure 2). However, there were no differences in the stem diameter of eucalyptus plants over the evaluated period (Figure 3).

Lower growth and death rates of apical meristems were observed, which are symptoms related to the mechanism of action of glyphosate and 2,4-D herbicides, directly impacting on the plant height increase (Oliveira Júnior, 2011). Plants in early growth stages tend to allocate most photoassimilates to primary growth, i.e., to height, with the aim of competing for light (Taiz and Zeiger, 2006), which may have influenced the height increase of eucalyptus plants subjected to

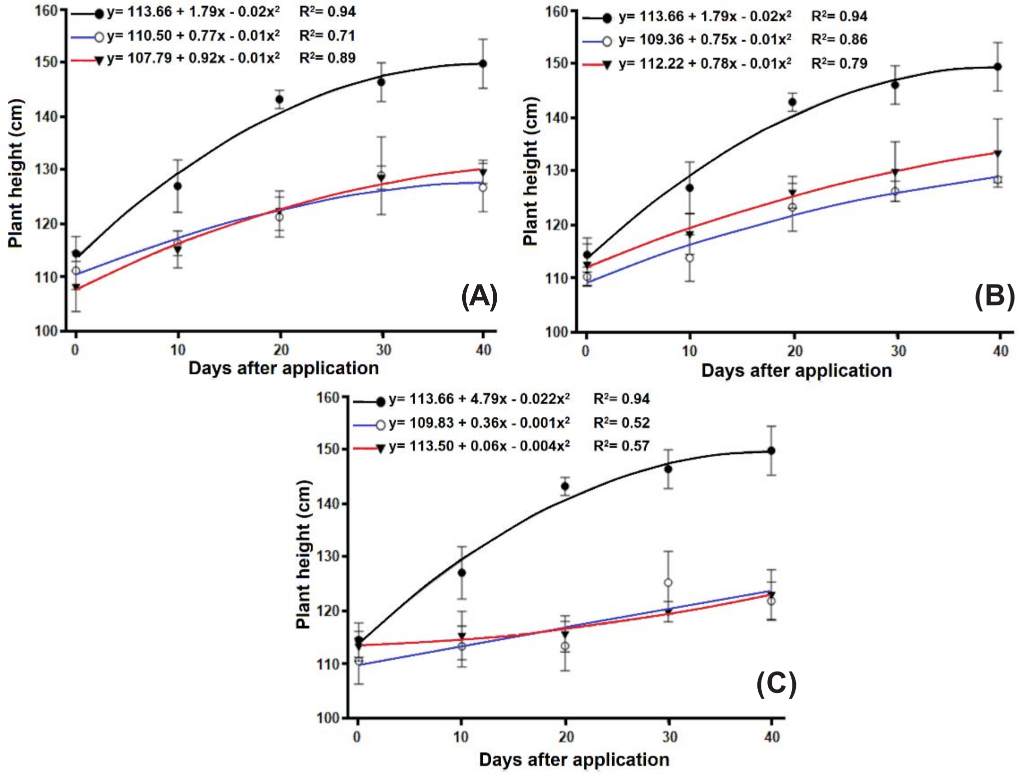

Figure 2 - Height (cm) of eucalyptus plants subjected to simulated drift of 2,4-D (A), glyphosate (B) and 2,4-D + glyphosate (C), with (—▼) and without (—○) Fertiactyl Pós® in comparison to the untreated control (—●).

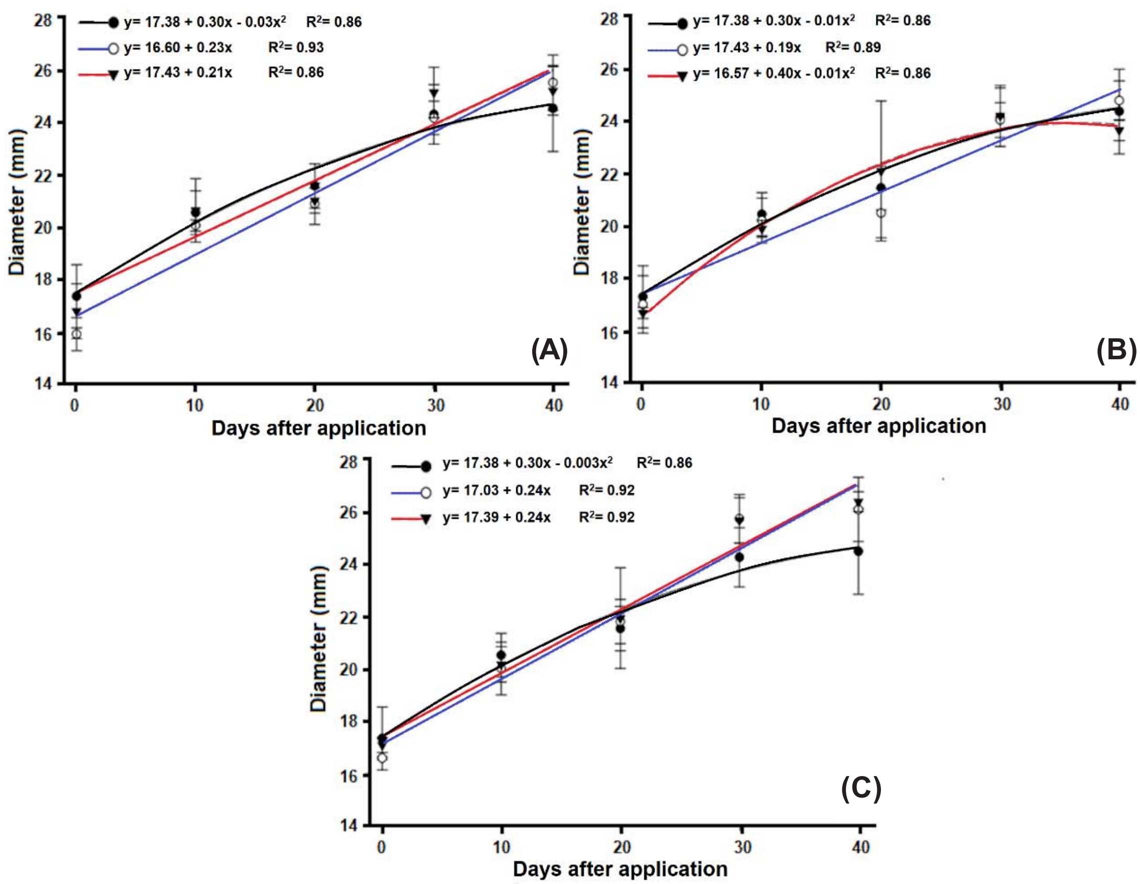

Figure 3 - Diameter of eucalyptus plants subjected to simulated drift of 2,4-D (A), glyphosate (B) and 2,4-D + glyphosate (C), with (—▼) and without (—○) Fertiactyl Pós® in comparison to the untreated control (—●).

drift compared to the control. Such an influence was not however, observed in the stem diameter. Similar results were observed in eucalyptus plants subjected to simulated drift of auxinic herbicides, with no differences in stem diameter being observed (Tuffi Santos et al. 2006; Carvalho et al. 2014). This fact may be related to the primary investment in height growth and the short period of time analyzed (45 and 50 DAA, respectively), similar to the period evaluated in the present study (40 DAA).

Eucalyptus leaf, stem and total shoot dry matter accumulation was affected by glyphosate, 2,4-D and their mixture, in the presence or absence of Fertiactyl Pós®, when compared to the untreated control. However, within each treatment where glyphosate and 2,4-D herbicides were not mixed in the tank, it was observed that, in the presence of Fertiactyl Pós®, eucalyptus plants accumulated higher biomass (Table 2). In a similar study, glyphosate and triclopyr drift reduced the dry matter accumulation of shoots of eucalyptus plants by 43.0 and 22.6%, respectively, while the mixture of these herbicides reducedthe dry matter by 59.6% (Tuffi Santos et al., 2006). The application of glyphosate + 2,4-D (180 + 335 g ha^{-1}) in mxture reduced the dry matter accumulation of *Ceiba pentandra* trees by 74.42% in comparison with the control. However, the isolated application of these herbicides reduced the dry matter accumulation by only 16.13 and 25.81%, respectively (Yamashita et al. 2009).

Table 2 **-** Dry matter mass of leaves (MSF), stem (MSC) and shoots (MSPA) of eucalyptus plants submitted to simulated drift of glyphosate, 2,4-D and 2,4-D + glyphosate + mixture, with and without Fertiactyl Pós®, and the untreated control at 40 days after application

Treatment	MSF	MSC	MSPA
	(g)		
2.4-D	133.9a*	124.8b*	258.7b*
2.4-D + Fertiactyl Pós®	139.8a*	137.0a*	276.8a*
Glyphosate	150.1b*	130.6b*	280.7b*
Glyphosate + Fertiactyl Pós®	154.7a*	141.5a*	296.2a*
Glyphosate + 2.4-D	132.8a*	129.5a*	262.3a*
Glyphosate + 2.4-D + Fertiactyl Pós®	124.6b*	127.1a*	251.7b*
Control	167.7	140.1	307.7

* Significant compared to the control by the Dunnett test at 5% probability. Averages followed by the same letter within each column do not differ at 5% probability by T test – plants with and without Fertiactyl Pós®.

The stress caused by herbicide application also reduced the leaf area of eucalyptus plants, leading to the lower biomass allocated to the formation of the photosynthetically active area, and culminating in a smaller AFE (Table 3). The lowest AFE was observed in eucalyptus plants treated with glyphosate + 2,4-D, regardless of the absence or presence of Fertiactyl Pós®, presenting reductions of by 25.2 and 24.1%, respectively, when compared to the untreated control (Table 3). The AFE reduction is reflected in the smaller amount of photosynthetic tissue and, consequently, in the lower photosynthetic rate per mass, thereby reducing the allocation of photoassimilates to plant tissues, causing less development and biomass accumulation in eucalyptus shoots (Table 2). The results of this study showed that the reduction in photosynthetic area of eucalyptus plants was influenced by the evaluated herbicides. The leaf ares of eucalyptus plants treated with glyphosate was 60% less when compared to untreated control plants (Pereira et al., 2011; Costa et al., 2012). On the other hand, the addition of increasing doses of Fertiactyl Pós® reduced the harmful effects of glyphosate drift on the leaf area of eucalyptus plants (Machado et al., 2017).

Glyphosate drift provides rapid increase in leaf blade thickness, which may be related to a plant response to the reduction in leaf area lost due to herbicide cell damage, resulting in a reduction of AFE (Tuffi Santos et al. 2005). However, among the symptoms related to auxinic herbicides are cell wall loosening and cell elongation due to fluid accumulation resulting from reduced osmotic potential (Silva et al., 2007), which in turn increases the AFE, as observed in the treatments of 2,4-D, both in the presence and absence of Fertiactyl Pós®.

Table 3 - Foliar area and specific foliar area of eucalyptus plants submitted to simulated drift of glyphosate, 2,4-D and 2,4-D + glyphosate + mixture, with and without Fertiactyl Pós®, and the untreated control at 40 days after application

Treatment	Foliar area (m^2)	Specific foliar area ($m^2\ kg^{-1}$)
2.4-D	1.33 a*	9.95 a
2.4-D + Fertiactyl Pós®	1.35 a*	9.67 a
Glyphosate	1.44 b*	9.60 a*
Glyphosate + Fertiactyl Pós®	1.47 a*	9.47 a*
Glyphosate + 2.4-D	1.03 a*	7.74 a*
Glyphosate + 2.4-D + Fertiactyl Pós®	0.98 b*	7.85 a*
Control	1.7	10.3

* Significant compared to the control by the Dunnett test at 5% probability. Averages followed by the same letter within each column do not differ at 5% probability by T test – plants with and without Fertiactyl Pós®.

Summarizing, it can be concluded that the addition of Fertiactyl Pós® to the herbicide syrup reduces the poisoning of eucalyptus plants drift-exposed to glyphosate and 2,4-D when these herbicides are applied in an isolated way. However, this protection does not occur for the glyphosate + 2,4-D mixture.

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