PHYSIOLOGICAL RESPONSE OF EUCALYPTUS SPECIES GROWN IN SOIL TREATED WITH AUXIN-MIMETIC HERBICIDES[1]

Resposta Fisiológica de Espécies de Eucalipto Cultivadas em Solo Tratado com Herbicidas Mimetizadores de Auxinas

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ABSTRACT - The objective was the assessment of the persistence and risk of intoxication of eucalyptus species by mixing picloram 2,4-D herbicide on application prior to forest planting. The experiment was deployed in the field in a completely randomized design with 6 repetitions, in a 4 x 5 factorial scheme, with four species (*Eucalyptus urophylla*, *E. globulus*, *E. saligna* and *Corimbia citriodora*) and five doses of the picloram base herbicide + 2,4-D (64 + 240 g L^{-1}): 0, 3, 4, 5, and 6 L ha^{-1} of the product, applied 30 days before deployment of culture. At 15 and 40 days after planting (DAP), physiological assessments were carried out. At 90 days after herbicide application (DAA) a bioassay took place to identify the residual effect of the product. No species showed visible symptoms of intoxication; however, there were physiological changes. For photosynthetic rate there were different responses, with reduction for larger doses, except in *E. saligna*. At 15 DAP, *E. saligna* and *E. urophylla* showed higher rates for stomatal conductance and transpiration. At 40 DAP, it was found that increasing the doses caused a linear decrease in stomatal conductance. Water use efficiency of plants was lower at 15 DAP in higher doses, with no changes at 40 DAP. Toxic symptoms were not noticed the in bioindicator at 90 DAA. However, increasing the applied doses caused a positive linear effect on the accumulation of biomass of the bioindicator. The mixture of picloram + 2,4-D did not cause morphological changes, but triggered negative physiological responses in the plants.

Keywords: *Eucalyptus*, picloram, 2,4-D, photosynthetic rate, water use efficiency, bioassay.

*RESUMO - Objetivou-se neste trabalho avaliar a persistência e o risco de intoxicação de espécies de eucalipto para mistura de herbicidas picloram + 2,4-D em aplicação prévia ao plantio florestal. O experimento foi implantado em campo, em delineamento inteiramente casualizado com 6 repetições, no esquema fatorial 4 x 5, com quatro espécies (**Eucalyptus urophylla**, **E. globulus, E. saligna** e **Corimbia citriodora**) e cinco doses de herbicida à base de picloram + 2,4-D (64 + 240 g L^{-1}): 0, 3, 4, 5 e 6 L ha^{-1} do produto, aplicadas 30 dias antes da implantação da cultura. Aos 15 e 40 dias após o plantio (DAP), foram feitas avaliações fisiológicas. Aos 90 dias após a aplicação do herbicida (DAA), foi realizado um bioensaio para identificar o efeito residual do produto. Nenhuma espécie apresentou sintoma visível de intoxicação, porém ocorreram alterações fisiológicas. Para taxa fotossintética, houve diferentes respostas, com redução para maiores doses, exceto em **E. saligna**. Aos 15 DAP **E. saligna** e **E. urophylla** apresentaram taxas superiores para condutância estomática e transpiração. Aos 40 DAP, constatou-se que o aumento das doses ocasionou diminuição linear na condutância estomática. A eficiência no uso da água das plantas foi menor aos 15 DAP nas doses mais elevadas, não havendo alterações aos 40 DAP. Não foram observados sintomas de intoxicação no bioindicador aos 90 DAA. Entretanto, o aumento das doses aplicadas ocasionou efeito linear positivo no acúmulo de biomassa do bioindicador. A mistura de picloram + 2,4-D não causou alterações morfológicas, porém desencadeou respostas fisiológicas negativas nas plantas.*

Palavras-chave: *Eucalyptus*, picloram, 2,4-D, taxa fotossintética, eficiência do uso da água, bioensaio.

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INTRODUCTION

Eucalyptus, in its different species cultivated in Brazil, has won a prominent place in the national forest sector, with about 5.1 million hectares of planted forests (ABRAF, 2013). The preferred choice by farmers for planting eucalyptus is related to its rapid growth, high forest productivity, mastering the production technology and the wide use of its wood.

In the cultivation of eucalyptus, one of the greatest difficulties in obtaining good crop yield refers to the availability of growth factors, almost always scarce in production environments. This fact is compounded by the presence of well adapted weeds to unfavorable edaphological climatic situations that are aggressive regarding the colonization of new areas and producing a high number of seedlings with a high potential for spreading and longevity.

The use of herbicides in weed management in planted forests is increasing, standing out in comparison to the other control methods. Glyphosate based herbicides have advantages such as low volatility and toxicity to mammals and aquatic life, and are also rapidly inactivated in soil; it is the most used active ingredient in most areas of eucalyptus plantations in Brazil. The continuous and repeated use of this molecule increases the selection pressure, with the possibility of change in the invasive flora, which has been selecting the plant species from the eudicotyledonous group, which are more tolerant to this product. In recent years, the frequency of these plants and their density in the areas have increased (Tuffi Santos et al., 2013) and, with it, concern with its handling and the number of interventions aimed at its control.

Increased doses of glyphosate within acceptable economic and environmental levels, and the use of alternative methods such as mowing, have burdened the activity of controlling weeds, and do not prove effective due to regrowth of the plants a few days after operations.

Herbicides based on 2,4-D and picloram have proven effectiveness in controlling eudicotyledonous weeds (Rodrigues & Almeida, 2005), including those considered tolerant to glyphosate (Robinson et al., 2012; Walker et al., 2012), becoming an alternative for its management. However, these herbicides have a high potential for leaching, with risk of contamination of groundwater aquifers (Dornelas de Souza et al., 2001; Berisford et al., 2006); furthermore, picloram has a long residual period (Close et al., 2003; D'Antonino et al., 2009). The physiological effect caused by these molecules on eucalyptus plants grown in areas with newly applied with 2,4-D and picloram is unknown.

In this study, the objective was the assessment of the persistence and risk of intoxication in eucalyptus species by mixing picloram 2,4-D herbicide on application prior to forest planting.

MATERIALS AND METHODS

Effect of 2,4-D + picloram on eucalyptus seedlings

The study was conducted in the March-July 2012 period, at the Institute of Agricultural Sciences of Universidade Federal de Minas Gerais, located in the Brazilian city of Montes Claros-MG, situated at longitude 43º 53' W, latitude 16º 43' S and 650 m altitude, being characterized by the Köppen classification as a Savanna Tropical climate (Aw), with hot and rainy summers and dry winters. Climatic data from the period of the test are shown in Figure 1.

The experiment was carried out in an open field with soil classified as Cambisol, whose physical and chemical characteristics are: a medium texture with 24% clay, 30% sand and 46% silt, having in the layer of 0 to 20 cm depth 14.96 mg kg^{-1} of P, 141.0 mg kg^{-1} of K, 7.50 $cmol_c$ dm^{-3} of Ca and 4.70 $cmol_c$ dm^{-3} of Mg, with a pH of 8.0.

The lineation employed was completely randomized with six replications in a 4 x 5 factorial design, with four species (*Eucalyptus urophylla, E. globulus, E. saligna* and *Corimbia citriodora*) and five doses of the mix of the picloram auxins + 2,4-D herbicides: 0 (control), 3, 4, 5, and 6 L ha^{-1} (commercial

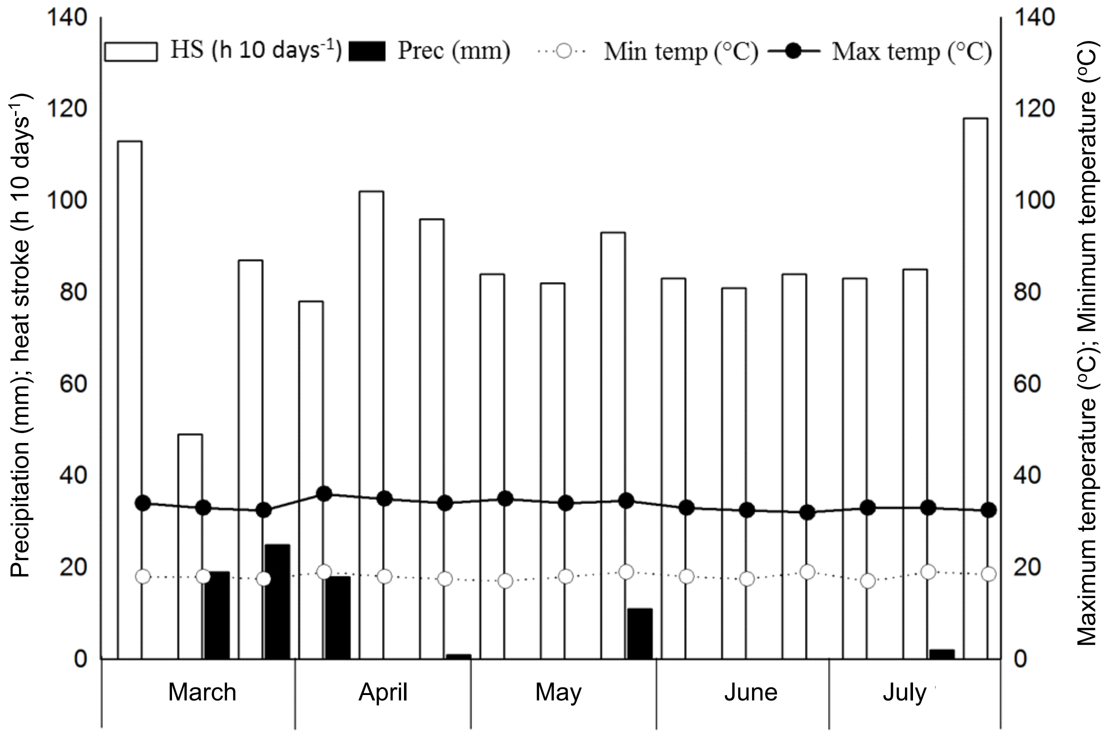


Figure 1 - Averages per decade of precipitation (mm), maximum temperature (°C), minimum temperature (°C) and heat stroke (h 10 days^{-1}) obtained while performing the experiment.

formulation Turuna@, corresponding to 240 g L^{-1} of 2,4-D + 64 g L^{-1} of picloram). The application was made on the ground, 30 days before crop deployment, with a backpack sprayer equipped with a cutting edge, model Teejet AI110015, and a constant pressure regulating valve at 150 kPa, with a spray adjusted volume of 100 L ha^{-1}. The application was carried out in the rows of the tree species in the range of 1.0 m of control.

The seedlings were grown in tubes of 55 cm^3, with a substrate composed of vermiculite, ground coal and cattle manure. Plants used in the assay were visually selected for size and vigor. They were transplanted in the experimental area into holes of 30 x 30 x 30 cm, spaced 3 x 3 m. The experimental plot had a seedling of eucalyptus, with six plants per treatment. The experimental area was kept weed-free with hand weeding after the emergence of the plants and irrigated by a sprinkler system, trying to keep the soil moist and seedling survival.

At 15 and 40 days after planting (DAP), which respectively represent 45 and 70 days after application (DAA) of the herbicide, physiological assessments were carried out using an infrared gas analyzer (IRGA), brand ADC, model LCA 4 (Analytical Development Co. Ltd, Hoddesdon, UK), in an open field with free circulation of air. The assessments were done in the upper third of the seedlings in a fully expanded leaf and seemingly without symptoms of intoxication, submitted to the analyzer using artificial lighting of 1,200 µmol m^{-2} s^{-1} in the camera of assessment of the equipment. For more homogeneous environmental conditions and accurate data reading, the analyzes were performed between 8 and 11 h.

Photosynthetic rate (A - µmol m^{-2} s^{-1}), stomatal conductance of water vapor (g_s - mol m^{-1} s^{-1}), transpiration rate (E - mol H_2O m^2 s^{-1}) and the concentration of CO_2 in the substomatal chamber (Ci - µmol mol^{-1}) were assessed, and was calculated the efficiency of the water use (WUE - mol CO_2 mol H_2O^{-1}), from the relationship of the photosynthesis by the amount of water transpired.

Data were subjected to analysis of variance by F test at 5% probability. In cases where there was interaction, the split was done with the comparisons between the means of the species, by Tukey test at 5% probability and the adjustment of regression equations based on the doses of herbicide applied, by testing the coefficients until 10% probability by the t test.

RESULTS AND DISCUSSION

Effect of 2,4-D + picloram on eucalyptus seedlings

The plants of *Eucalyptus urophylla, Eucalyptus saligna, Eucalyptus globulus* and *Corimbia citriodora* transplanted at 30 days after applying 2,4-D + picloram in clay soil showed no visible sign of intoxication during the 70 days of cultivation of seedlings, although the plant physiology has been altered. It is noteworthy that physiological variables are important indicators of the action of herbicides and may show changes without exhibit the visual symptoms, such as in the present study.

At 15 and 40 days after planting the culture (DAP), there was an interaction between the dose and species factors in relation to the photosynthetic rate; the plants of the species *E. urophylla* and *E. saligna* assessed at 15 DAP and *E. saligna* at 40 DAP were not influenced by the dose of the mix 2,4-D + picloram (Figure 2A, B). At 40 DAP, *E. urophylla* showed a marked decrease in this variable for plants grown in soil treated with doses higher than 5 L ha^{-1} of the herbicide (Figure 2B). Seedlings of *E. globulus* showed a linear decrease in photosynthetic rate, with increasing doses of herbicide in the two assessments performed, despite the low adjustment of the equation for this species (Figure 2A, B).

As for *C. citriodora*, a quadratic behavior for photosynthetic rate as a function of the doses of 2,4-D + picloram was observed. For this species, lower residue levels stimulate photosynthesis in plants, whereas higher doses at 4 L ha^{-1} decrease the photosynthetic rate in the initial phase of seedling growth (Figure 2A, B).

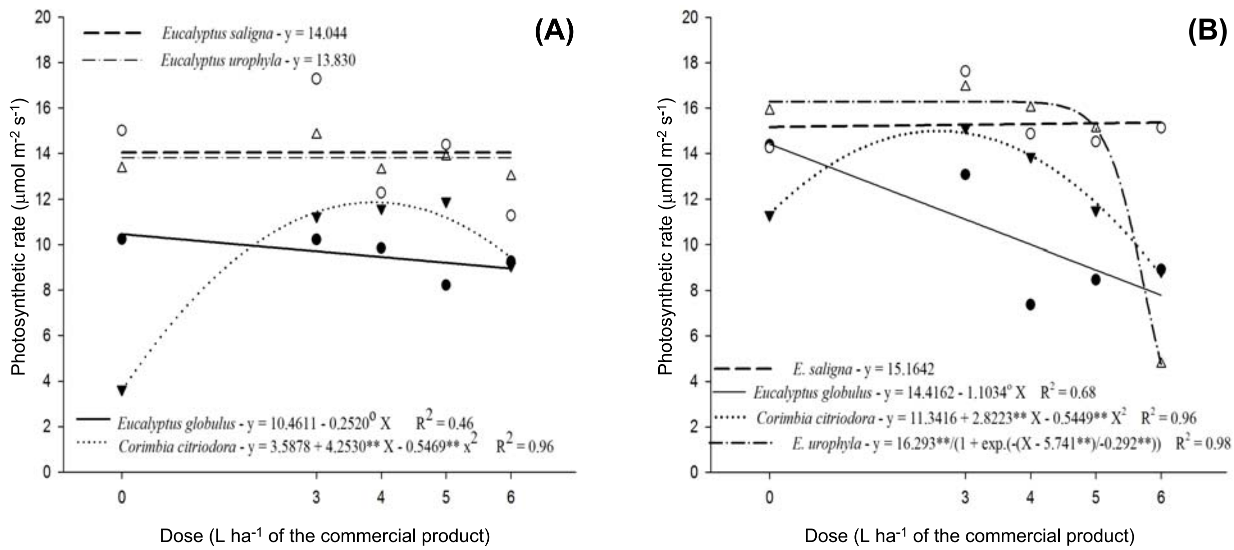
At 15 DAP, *E. saligna* presents a higher photosynthetic rate than the other species in the absence of herbicide in soil or application of 3 L ha^{-1} of the herbicide mixture (Table 1). In these same doses there is no difference in this variable between the species in the assessment done at 40 DAP. *E. saligna* and *E. urophylla* were, among the species, those most photosynthetically active at all doses applied at 15 DAP and in most of the doses assessed at 40 DAP (Table 1).

Stomatal conductance (g_s) assessed at 15 DAP was not influenced by the doses of the herbicide nor by the interaction between the dose versus species factors. However, in the assessment at 40 DAP this characteristic was influenced by two factors; the increase of the doses of the product caused a linear decreased in g_s (Figure 3). Higher values of g_s were observed for *E. saligna,* followed by *E. urophylla* (Table 2).

With the expansion of the eucalypt root system due to the development of seedlings between assessments, the most contact with the herbicide may have occurred and, consequently, higher absorption, which would explain the effect of increasing doses in reducing g_s of the species of eucalyptus assessed at 40 DAP. Some hormonal herbicides stimulate the production of ethylene (Mercier, 2004; Machado et al., 2006) and, consequently, the synthesis of the abscisic acid, which, after being translocated, operates in stomatal closure (Mercier, 2004) and negatively affects g_s.

The photosynthesis and transpiration are influenced by the flow of gases in the cell (Messinger et al., 2006), which in turn depends on the stomatal opening (Taiz & Zeiger, 2004). This mechanism may explain the fact that *E. saligna* and *E. urophylla* present themselves among the most photosynthetically active species (Table 1), as they have also obtained high values of g_s. The behavior of the species indicates a clear relationship between the g_s and the photosynthetic response.

The photosynthesis can also be limited by changes in gene expression of plants intoxicated with auxin herbicides; these products can block or maximize the expression of some genes and change the rates of



** and ° the significant at 1 and 10% by the t test.

Figure 2 - Photosynthetic rate in seedlings of four species of eucalyptus transplanted 30 days after applying the commercial mixture 2,4-D + picloram (240 + 64 g L^{-1}), at 15 (A) and 40 (B) days after transplantation.

Table 1 - Photosynthetic rate in seedlings of four species of eucalyptus transplanted 30 days after applying the commercial mixture 2,4-D + picloram (240 + 64 g L^{-1}), at 15 and 40 days after transplantation

15 days after planting					
Species	Doses L ha^{-1}				
	0	3	4	5	6
Corimbia Citriodora	3.59 c	11.21 ab	11.59 a	11.91 ab	9.07 b
Eucalyptus globulus	10.24 b	10.22 c	11.41 a	8.16 b	9.26 ab
Eucalyptus saligna	15.01 a	17.27 a	13.01 a	14.32 a	11.27 ab
Eucalyptus urophylla	13.48 ab	14.82 ab	13.61 a	13.86 a	12.99 a
CV (%)	20.04				
40 days after planting					
Corimbia Citriodora	12.19 a	15.13 a	13.86 a	11.51 ab	9.18 b
Eucalyptus globulus	14.38 a	13.08 a	7.89 b	8.47 b	8.93 b
Eucalyptus saligna	14.38 a	17.62 a	14.88 a	14.53 a	16.56 a
Eucalyptus urophylla	15.89 a	16.98 a	16.01 a	15.12 a	3.97 b
CV (%)	24.80				

Means followed by the same letters in the column do not differ among themselves by the Tukey test ($p < 0.05$).

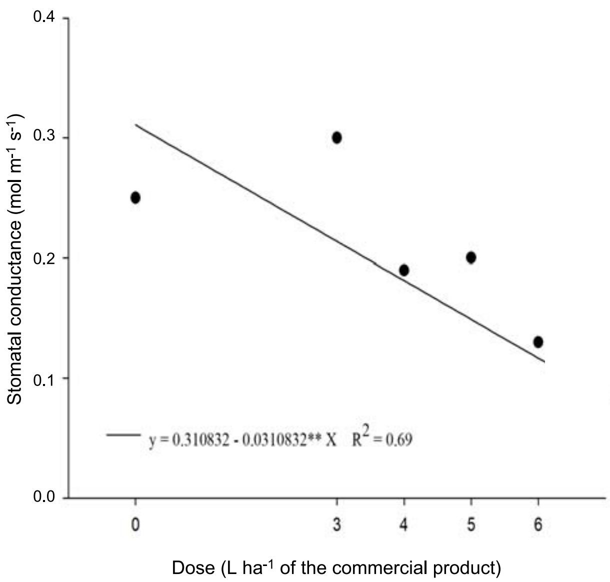
synthesis of enzymes essential to the photosynthetic process and the metabolism of other cellular components (Machado et al., 2006; Chapman & Estelle, 2009). The response at 15 and 40 DAP of *C. citriodora* may be related to this situation, since the photosynthetic result of the species was positively influenced in smaller doses and negatively in larger ones (Figure 2A, B). The auxin-mimetic can act as growth stimulants in plants, specially when they are found in low levels in the soil (Cedergreen et al., 2007; Calabrese & Blain, 2009).

The transpiration assessed at 15 DAP showed interaction among the species and the doses of 2,4-D + picloram, in which *E. saligna* and *E. globulus* did not change their behavior due to the application of the herbicide and were more tolerant to the chemical action of the product for this factor (Figure 4A). *E. urophylla* and *C. citriodora* showed initial increase in transpiration rate (E), with further reduction from the commercial dose of 4 L ha^{-1}. The transpiration assessed at 40 DAP was not influenced by the species factor, without interaction occurrence in this period (Table 2), but increasing doses of herbicide caused the uniform behavior of the species, with a linear reduction of E (Figure 4B).

By analyzing the interaction that occurred at 15 DAP, it may be realized that leaf transpiration was lower in *C. citriodora* in the absence of herbicide in the soil, with no variation between the other species (Table 3). Plants grown in soil treated with doses greater than 4 L ha^{-1} of the herbicide showed no difference between species in transpiration (Table 3). *E. saligna* and *E. urophylla* showed higher values of E at all doses of the herbicide (Table 3).

The regulation of opening and closing of stomata influences transpiration: the smaller the opening of stomata, the lower the E due to higher stomatal resistance. The g_s regulates the input flow of CO_2 and water output by the stomata (Taiz & Zeiger, 2004).

The auxins herbicides adversely affect the root system, where there is increase in cellulases that cause the deterioration of the roots in sensitive species (Silva et al., 2007); consequently, the is reduction in water



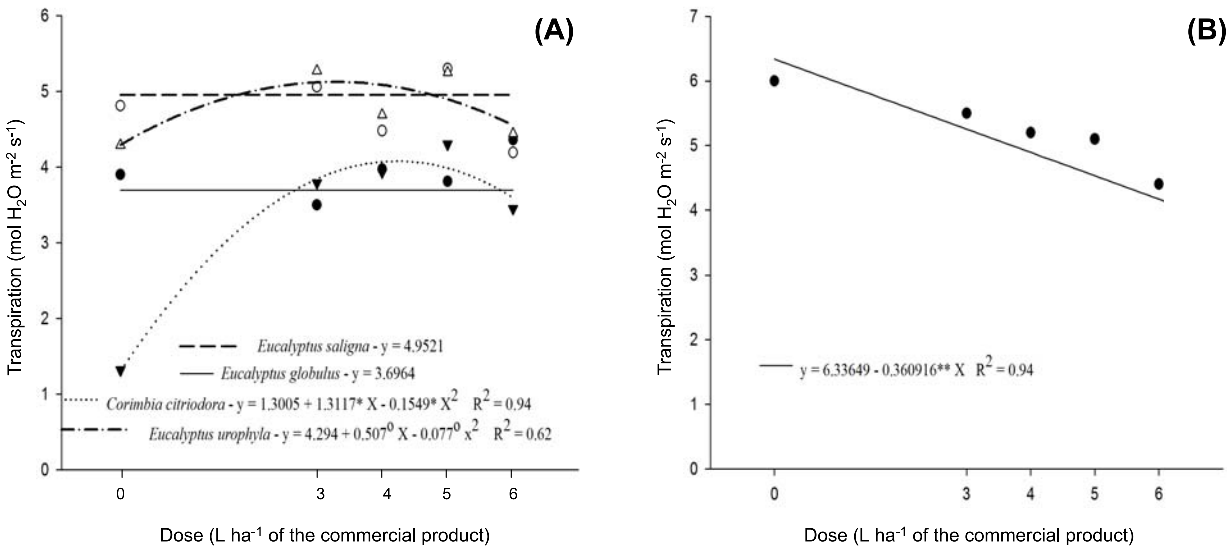
** and º significant at 1 and 10 % by the t test.

Figure 3 - Stomatal conductance (g_s) in seedlings of four species of eucalyptus transplanted 30 days after applying the commercial mixture 2,4-D + picloram (240 + 64 g L^{-1}), at 40 days after transplantation.

Table 2 - Stomatal conductance (g_s), transpiration (E), internal carbon of the leaf (Ci) and efficiency of the water use (WUE) in seedlings of four species of eucalyptus transplanted 30 days after applying the commercial mixture 2,4-D + picloram (240 + 64 g L^{-1}), at 15 and 40 days after transplantation

Physiological assessments at 15 days after planting				
Species	g_s	E	Ci	WUE
Corimbia Citriodora	0.155 b	–	210.39 b	2.841 ab
Eucalyptus globulus	0.169 b	–	237.30 a	2.513 b
Eucalyptus saligna	0.226 a	–	215.73 b	3.019 a
Eucalyptus urophylla	0.219 a	–	219.78 ab	2.899 a
CV (%)	32.12	–	11.33	18.04
Physiological assessments at 40 days after planting				
Corimbia Citriodora	0.173 b	4.840 a	219.08 b	2.68 a
Eucalyptus globulus	0.186 b	4.849 a	238.44 a	2.23 a
Eucalyptus saligna	0.262 a	5.774 a	215.04 b	2.77 a
Eucalyptus urophylla	0.227 ab	5.554 a	218.88 b	2.45 a
CV (%)	40.42	26.93	9.61	34.01

Means followed by the same letters in the column do not differ among themselves by the Tukey test ($p < 0.05$).



** and º significant at 1 and 10 % by the t test.

Figure 4 - Transpiration in seedlings of four species of eucalyptus transplanted 30 days after applying the commercial mixture 2,4-D + picloram (240 + 64 g L^{-1}), at 15 (A) and 40 (B) days after transplantation.

Table 3 - Transpiration (E) in seedlings of four species of eucalyptus transplanted 30 days after applying the commercial mixture 2,4-D + picloram (240 + 64 g L^{-1}), at 15 days after transplantation

Physiological assessments at 15 days after planting					
Species	Doses L ha^{-1}				
	0	3	4	5	6
Corimbia Citriodora	1.34 b	3.83 bc	3.93 a	4.30 a	3.45 a
Eucalyptus globulus	3.90 a	3.50 c	4.58 a	3.78 a	4.16 a
Eucalyptus saligna	4.81 a	5.05 ab	4.49 a	5.27 a	4.19 a
Eucalyptus urophylla	4.31 a	5.28 a	4.84 a	5.25 a	4.44 a
CV (%)	21.26				

Means followed by the same letters in the column do not differ among themselves by the Tukey test ($p < 0.05$).

absorption by the root system. This decrease leads to cell sagging, including the guard cells, as a function of the difference in turgor to other cells of the epidermis or its subsidiaries and, consequently, the stomatal closure and reduction of water losses (Larcher, 2000).

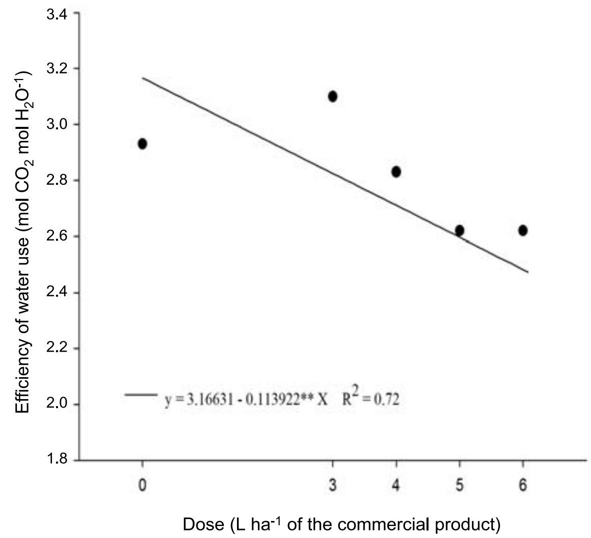
The concentration of CO_2 in the the substomatal chamber (Ci) was not altered by the action of the herbicide in both assessments performed. However, plants of *E. globulus* obtained the highest concentrations at 15 and 40 DAP (Table 2).

The stomatal closure triggers a reduction in the inner CO_2 levels, directly affecting the activity of Rubisco (ribulose-1.5-bisphosphate carboxylase/oxygenase) and, consecutively, the photosynthetic rate (Taiz & Zeiger, 2004). The concentration of CO_2 in the leaf internal space is dependent on the photosynthetic activity, which, by carboxylation, incorporates CO_2 for the formation of organic compounds. Among different species may be those with more stomatal resistance to the absorption of CO_2, reducing the internal concentration of this compound (Da Matta et al., 2001). This mechanism leads to the fact that *E. globulus* has high concentrations of Ci at 15 and at 40 DAP (Table 2), since this species has shown low photosynthesis rate in these assessments (Figure 2A, B) and, therefore, little incorporation of CO_2 while maintaining high Ci.

This process of carboxylation efficiency is limited mainly by factors such as the very availability of CO_2 and the behavior of the enzyme activity (Larcher, 2000). When the CO_2 is totally converted, the process then becomes limited by the atmosphere and not by the plant (Larcher, 2000); it is evident that, for *E. globulus*, the lowest accumulation of CO_2 is directly related to the limitation of photosynthesis, in addition to intrinsic features of the physiology of the species.

The water use efficiency (WUE) at 15 DAP was influenced by the herbicide in the soil, with a linear reduction of the observed values versus the increase of the applied doses (Figure 5). *E. urophylla* and *E. saligna* showed higher WUE at 15 DAP, specially regarding *E. globulus* (Table 2). However, at 40 DAP there was no effect of dose or species on this variable.

The greater efficiency of water use is related to the shorter opening of the stomata, since this opening provides CO_2 uptake for photosynthesis and water loss through transpiration (Pereira Netto et al., 2002). This shows that, at 15 DAP, plants of *E. urophylla* and *E. saligna* obtained a better stomata control. However, at 40 DAP there is no effect caused by the application, as well as difference in the response among plants. With the highest residual herbicide in the soil, *E. globulus* behaves as a species of least efficient use of this resource.



** and º significant at 1 and 10 % by the t test.

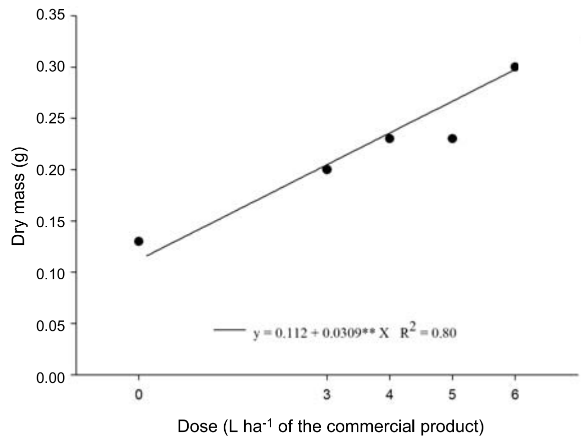
Figure 5 - Efficiency of water use in seedlings of four species of eucalyptus transplanted 30 days after applying the commercial mixture 2,4-D + picloram (240 + 64 g L^{-1}), at 15 days after transplantation.

Residual action of 2,4-D + picloram in a soil cultivated with eucalyptus

Visually, the cucumber plants grown in soil treated with picloram + 2,4D showed no signs of intoxication at 90 days after application of the mixture, but a positive linear effect on biomass accumulation by cucumber because of increasing doses of herbicide (Figure 6). Symptoms of intoxication produced by the leaves of eudicotyledonous species by auxins herbicides are easily characterized (Thill, 2003).

Some herbicides used in sub-doses may exert an inverse function and assist in plant development (Cedergreen et al., 2007; Silva et al., 2009). In larger doses, there was a stimulatory effect of the auxin herbicide. The reaction that occurred can be called hormesis; the phenomenon is studied mainly in relation to the mechanism of plants response, which can result in gains for the species (Cedergreen, 2008; Calabrese & Blain, 2009). However, this fact suggests the presence of the mixture of picloram + 2,4-D in soil at 90 days after applying the product, due to the increase of cucumber biomass.

The behavior of herbicides in the environment can be influenced by physicochemical and microbiological characteristics of the soil, environmental factors and the properties of the herbicide. The persistence of picloram in soils is considered moderate to high (Dornelas de Souza et al., 2001; Berisford et al., 2006; Procópio et al., 2008), ranging from 20 to 300 days (Fairchild et al., 2009), with a half-life of 90 days (Rodrigues & Almeida, 2005). As 2,4-D shows persistence of short to medium, reaching approximately 50 days (Rodrigues & Almeida, 2005), the results observed at 90 DAA therefore indicate a greater influence of



** significant at 1 % by t test.

Figure 6 - Dry mass of cucumber grown in clay soil at 90 days after applying the commercial mixture of 2,4-D (240 g L^{-1}) + picloram (64 g L^{-1}).

the proportion of picloram present in the mixture.

The texture, organic matter and soil pH affect the picloram residual in the atmosphere (D'Antonino et al., 2009), increasing or decreasing its permanence. The soil used in this study has a high clay and organic matter content, besides pH equal to 8.0, features that may have influenced the absence of visible symptoms on eucalyptus species.

Picloram has high solubility in water (Close et al., 2003) and, with this high pH, the product has less sorption on clay particles (D'Antonino et al., 2009), being thus more readily leached and more available to microbiological degradation. According to studies from Berisford et al. (2006), picloram has high mobility both laterally and vertically in the soil. The degradation by the microbiota of the soil and leaching to deeper layers of its profile may have contributed to reduce the amount of herbicide available for absorption by the roots of eucalyptus seedlings, and does not cause visual damage to the plants of interest. It is noteworthy that in other soil and environment conditions, or even for other crops sensitive to auxin-mimetic, the results may be contrasting. In sandy-loamy soil, the presence of picloram was found even after 360 days from the application, with characteristic symptoms of toxicity observed in cucumber plants (Santos et al., 2006).

The cultivation of eucalyptus species in soils previously treated with picloram + 2,4-D should be assessed with caution, given the characteristics of high persistence of picloram in certain soils, especially in sandy soils with low organic matter content (Santos et al., 2006; D'Antonino et al., 2009).

Bioassays with picloram and 2,4-D are made by using very sensitive bioindicator plants, such as cucumber. As plants react differently to farming on soil treated with auxin-mimetic herbicides (Santos et al., 2013), the absence of visible injuries in eucalyptus suggest increased tolerance to these herbicides. However, field studies in different soil and environments types should be performed in order to assess the effects of auxin-mimetic growth and productivity of forest populations.

During the experiment, no eucalyptus species presented visual symptoms of intoxication. However, the residual herbicide in the soil affects the plants physiologically as a response to the action of the herbicide. Higher doses of the mixture picloram + 2,4-D provided reductions in most physiological variables of the species tested, which demonstrates the fragility of the seedlings to the herbicide and the importance of these assessments on the response of plants to herbicides. Physiological changes are dependent on the dose of herbicide, forest species and time after application. The bioassay with cucumber visually showed no intoxication of the plants, even though the dry mass has increased with increasing doses of the commercial mixture of 2,4-D (240 g L^{-1}) + picloram (64 g L^{-1}) in clay soil.

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