GROWTH ANALYSIS OF HYBRID *Eucalyptus urograndis* (*E. grandis x E. urophylla*) IN RESPONSE TO SIMULATED DRIFT OF AUXINIC HERBICIDES[1]

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ABSTRACT – The aim of this study was to evaluate growth rates of *E. urograndis* under application of triclopyr and fluroxipyr+triclopyr herbicides. Was used a randomized block design with four replications in a factorial design (3x2x5), corresponding to herbicides (triclopyr and fluroxipyr+triclopyr), both with two doses (0.75 and 1.5% concentrations of 480 g and L^{-1} for triclopyr and 80+240 g and L^{-1} for fluroxipyr+triclopyr), plus one control (plants that did not receive herbicide application) and five evaluation dates at 15, 30, 45, 60 and 75 days after application (DAA). At 45 days after seedlings transplant, applications were performed with pressurized carbon dioxide backpack sprayer, equipped with beak tip (XR 110.02) with spray volume of 200 L ha^{-1} and constant pressure of 35 kgf/cm^2. Were evaluated: leaf area (LA), specific leaf area (SLA), leaf area ratio (LAR), leaf weight ratio (LWR), absolute growth ratio (AGR), relative growth ratio (RGR) and net assimilatory ratio (NAR). The drift of triclopyr and fluroxipyr + triclopyr herbicide in the hybrid *E. urograndis* affect plant development in the beginning of the establishment. At 75 days after simulation drift of herbicides the plants showed characteristics of the symptoms of intoxication. It's necessary to perform studies after 75 DAA to verify the complete recovery of the intoxication symptoms of *E. urograndis* hybrid plants resulting from the herbicide drift in the present study.

Keywords: Weed competition; Reforestation; Chemical control.

ANÁLISE DE CRESCIMENTO DO HÍBRIDO Eucalyptus urograndis (E. grandis x E. urophylla) EM RESPOSTA À DERIVA SIMULADA DE HERBICIDAS AUXÍNICOS

RESUMO – O objetivo deste estudo foi avaliar índices de crescimento do híbrido de ***Eucalyptus urograndis*** *sob aplicação dos herbicidas triclopyr e fluroxipyr+triclopyr. Utilizou-se delineamento em blocos casualizados com quatro repetições em esquema fatorial (3x2x5), correspondendo aos herbicidas (triclopyr e fluroxipyr+triclopyr), ambos em duas doses (0,75 e 1,5% da concentração de 480 g.e.a* L^{-1} *para triclopyr e 80+240 g.e.a* L^{-1} *para fluroxipyr+triclopyr) mais uma testemunha (plantas que não receberam aplicação dos herbicidas) e cinco datas de avaliação aos 15, 30, 45, 60 e 75 dias após aplicação (DAA). Aos 45 dias após o transplante das mudas, foram realizadas aplicações com pulverizador costal pressurizado a gás carbônico, equipado com pontas bico (XR 110.02) com volume de calda de 200 L* ha^{-1} *e pressão constante de 35* kgf/cm^2*. Foram avaliados: área foliar (AF), área foliar específica (AFE), razão de área foliar (RAF), razão de peso de folha (RPF), taxa de crescimento absoluto (TCA), taxa de crescimento relativo (TCR) e taxa assimilatória líquida (TAL).*

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*A deriva dos herbicidas triclopyr e fluroxipyr+triclopyr no híbrido **E. urograndis** prejudica o desenvolvimento das plantas no início do estabelecimento. Aos 75 dias após a simulação da deriva dos herbicidas as plantas apresentam características de recuperação dos sintomas de intoxicação. É necessário a realização de estudos superiores a 75 DAA para comprovar a recuperação total dos sintomas de intoxicação de plantas do híbrido de **E. urograndis** resultante da deriva dos herbicidas em estudo.*

Palavras-Chave: Matocompetição; Reflorestamento; Controle químico.

1. INTRODUCTION

In 2012, Eucalyptus planted areas totaled 5 million hectares, representing growth of 4.5% (228 thousand hectares) compared to the 2011 indicator. The establishment of new plantations is related to future demand for industrial projects in the pulp and paper sector that was the main responsible for the accelerated growth of new areas (Associação Brasileira de Produtores de Florestas Plantadas, 2013). According to the growing demand for wood products, knowledge about weed management and the effect of herbicide drift on commercial reforestation areas has attracted attention from several researchers in recent years (Takahashi et al., 2009; Tiburcio et al., 2012, Carvalho et al., 2014).

Although the genus Eucalyptus presents fast-growing species of high competitiveness in terms of its establishment in the field, this does not exempt it from the interference of weeds, in addition, these plants may impair the growth and development of the crop due to water competition, nutrients and light, mainly in the initial period of development, and therefore, the quantitative and qualitative decrease of its production is observed as a consequence (Souza et al., 2003; Takahashi et al., 2009; Agostinetto et al., 2010) .

Among the possibilities of weed management, the chemical method is the most used, given the cost and the necessary manpower. Due to the scarcity of registered products for eucalyptus crop for selective control in post-emergence of weeds, the use of chemical control should be very cautious avoiding injury and productivity losses due to the deposition of unwanted drops in the plants of interest (Tiburcio et al., 2012). However, when the herbicide is not selective, it should be applied in a protected manner, since the deposition of the product outside the target can cause serious damage to the plants of interest (Santos et al., 2006).

Currently, eucalyptus cultivation has intensified in the cerrado regions, with emphasis on the states of Minas Gerais, Bahia and Tocantins, however, these newly exploited areas regenerate high-vigor plants, commonly known as cerrado regrowths, which require specific control methodologies (Associação Brasileira de Produtores de Florestas Plantadas, 2013).

Thus, herbicides commonly used to control weeds in pastures, such as auxinic herbicides, have been tested mainly in the control of sprouts of cerrado plants, with possible extension for use in reforestation with eucalyptus. Auxinic herbicides as well as triclopyr and the formulated fluroxipyr + triclopyr mixture induce changes in the metabolism of nucleic acids and proteins, interfering with the action of the RNA polymerase enzyme (Thill, 2003). Therefore, the application of these herbicides can induce intense cell division in tissues, causing leaf and stem epinasty, as well as, interrupt the normal flow of photoassimilates from the leaves to the root system (Silva et al., 2007). Thus, higher concentrations of these herbicides cause disorganized plant development, as well as stem deformation, callus formation and thickening of the terminal buds (Zimdahl, 1999; Yamashita et al., 2009).

Therefore, knowing the effects of drift of these herbicides on eucalyptus is essential for these products to be well used in the management of weeds in commercial reforestation.

Carvalho et al. (2014) report the effect of the simulated drift of auxinic herbicides on the initial development of eucalyptus seedlings, which showed a reduction in growth in height, diameter and accumulation of biomass at 28 days after application, with herbicides and doses tested, the effect of drift is less pronounced when applied to the herbicides triclopyr and fluroxypyr + triclopyr, both at 25% of the recommended dose.

Considering the lack of results regarding the development, tolerance and/or sensitivity to auxin mimetic herbicides in commercial clones of *Eucalyptus urograndis* hybrid (*E. urophylla* x *E. grandis*), the aim of this research was to evaluate the effects of drift simulated in the growth analysis of the hybrid *E. urograndis* with the herbicides triclopyr and fluroxipyr + triclopyr.

2. MATERIAL AND METHODS

The study was conducted under field conditions at the experimental station of the Federal University of Tocantins (UFT), Campus of Gurupi (11º 43 'S and 49º 04' W, at 280 m altitude). The local climate according to the classification of Köppen is tropical savanna (Aw), and humid with small water deficiency in the winter, megathermal with evapotranspiration concentration in the summer less than 48% of the annual total (B1wA'a'), according to the Thornthwaite classification (Peel et al., 2007). The annual average temperature is 27ºC and average annual precipitation of 1.600 mm, being rainy summer, dry winter and high water deficit between the months of May to September.

The experiment was developed in PVC pipes with dimensions of 30 x 40 x 30 cm, totaling a volume of 35 l, arranged in spacing of 1,5 x 1,5 m in the experimental area. The soil used to fill the tubes was collected in the arable layer of the experimental farm of the Federal University of Tocantins, characterized as a dystrophic Red - Yellow Latosol, medium texture, being a typical cerrado soil (Empresa Brasileira de Pesquisa Agropecuária, 2006), whose chemical attributes expressed in $cmol_c dm^{-3}$ (except pH) were: Ca = 4.3, Mg = 2.6, Al = 0.1, H + Al = 3.3 and K = 0.6; pH in H_2O = 5.8 and $CaCl_2$ = 5.0 and physical attributes: Sand = 76%, silt = 6% and clay = 18%.

Seedlings of the *E. urograndis* (*E. urophylla* x *E. grandis*) hybrid, approximately 30 cm high and three months old, were transplanted into the tubes, which received fertilization with the formulation 5-25-15 of NPK (13 g $tube^{-1}$). These were kept in the open during all the conduction of the experiment.

At 45 days after transplanting, when the plants were about 50 cm high, the applications were carried out with carbonic pressurized costal sprayer equipped with tips nozzle (XR 110.02) with a of spray volume of 200 L ha^{-1} and constant pressure of 35 kgf cm-^{2}. The application was made in order to reach the entire canopy of the plant. To avoid contact of the herbicides with the control (plants that did not receive herbicide application), they were protected during the application of the treatments.

A randomized block design with four replicates in a factorial scheme (2 x 2 x 5) + 1, corresponding to the herbicides (triclopyr and fluroxypyr + triclopyr), both in two doses (0.75 and 1.5% of the concentration of 480 g L^{-1} for triclopyr and 80 + 240 g L^{-1} for fluroxypyr + triclopyr), and five evaluation dates at 15, 30, 45, 60 and 75 days after application (DAA) and one control (plants not receiving herbicides), totaling 120 tubes (Table 1).

Table 1 – Treatments evaluated and the respective amounts of active ingredient, mineral oil and commercial product.

Tabela 1 – *Tratamentos avaliados e as respectivas quantidades de ingrediente ativo, óleo mineral e produto comercial.*

Tratamentos		Oil (L/ha)	Dose (% g.e.a.)	Dose c.p. (ml)
Herbicidas	Concentration (g.e.a L^{-1})			
Control	—-	—-	—-	
Triclopyr	480	1	0.75	7.5
Triclopyr	480	1	1.5	15
Fluroxipyr + triclopyr	80+240	1	0.75	15
Fluroxipyr + triclopyr	80+240	1	1.5	30

g.e.a. = grams of the herbicide contained in the commercial formula; **C.p.** = Commercial product
[1]**Triclopyr** = Common name of the acid equivalent contained in the herbicide Garlon 480 BR®;
[2]**Fluroxipyr + triclopyr** = common name of the acid equivalent contained in herbicida Truper®.

At 15, 30, 45, 60 and 75 DAA, four plants of each treatment, including the control, were harvested with the aerial part and root, separated and conditioned in paper bags, kept in an air circulation oven (70 ± 2 ° C) until reaching constant weight.

In order to determine leaf area (LA), net assimilation rate (NAR), relative growth rate (RGR), and absolute growth rate (AGR), and before obtaining data of leaf area, leaf dry mass and total were calculated: Specific leaf area (SLA), leaf area ratio (LAR), leaf weight ratio (LWR) as recommended by Benincasa (2003):

Since it is a non-additive model and consists of quantitative data, the most appropriate way to treat growth data along the plant ontogeny should be graphs and the discussion was based on the analysis of the trend of growth curves (Radford, 1967; Barreiro et al. (2006).

3. RESULTS

The leaf area plants of *E. urograndis* treated with herbicide increased over time, however, when compared

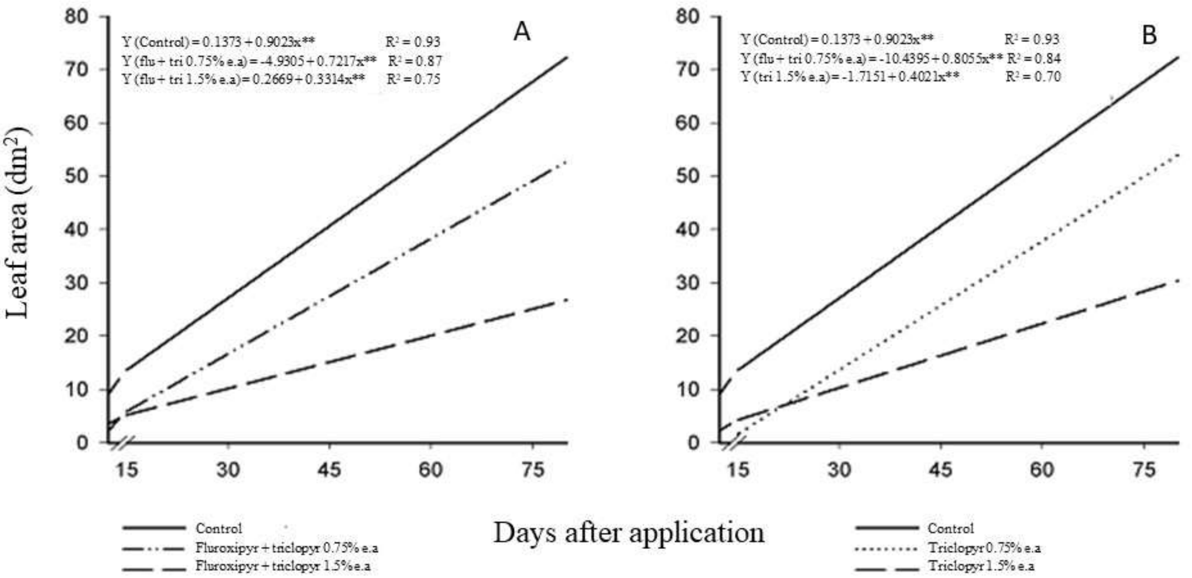

Figure 1 – Leaf area (dm^2) of *E. urograndis* hybrid in function of time, in response to application of the triclopyr (0.75 and 1.5% e.a.) and fluroxipyr + triclopyr herbicides (0.75 and 1.5% e.a.) - (tri - triclopyr; flu + tri - fluroxipyr + triclopyr; e.a. - equivalent acid). Gurupi - TO. ** ($p < 0.01$).

Figura 1 *– Área foliar (dm^2) do híbrido* ***E. urograndis*** *em função do tempo, em resposta a aplicação dos herbicidas triclopyr e fluroxipyr+triclopyr (0,75 e 1,5% e.a.) - (tri - triclopyr; flu+tri - fluroxipyr+triclopyr; e.a. - equivalente ácido). Gurupi - TO, 2014.** (p<0,01).*

to the control, the treatments showed a reduction, adjusting to the significant linear regression model ($p \leq 0.01$) with high determination coefficients (R^2).

Comparing the linear coefficients of the equations, it was verified that the effect of the herbicides was more accentuated at the doses of 1.5% e.a. (Figure 1).

It was observed that the SLA of hybrid plants treated with herbicides was superior to the control at 45 DAA, during which time the plants showed detoxification, with leaf exchange. The plants exposed to fluroxipyr + triclopyr 0.75% e.a at 60 DAA presented mean values higher than the control, being in the order of 0.2820 $dm^2\ g^{-1}$, while the control presented mean values of 0.2321 $dm^2\ g^{-1}$. It was observed maturation of the new leaves at 75 DAA, while the detoxification of the plants was verified, resulting in the reduction of the SLA due to the increase of the leaf thickness (Figure 2A).

Plants of the *E. urograndis* hybrid treated with auxinic herbicides presented decrease of LAR in the period between 15 and 30 DAA, which is explained due to the initial toxic effect of herbicides on the development of sensitive plants. The maximum LAR (0.0764 $dm^2\ g^{-1}$) was obtained in the treatment fluroxipyr + triclopyr 0.75% e.a. at 60 DAA (Figure 2B).

At 75 DAA, maximum values were obtained in all treatments of the leaf weight ratio (RPF), being greater in the treatment fluroxipyr + triclopyr 1.5% and (0.8111 $g.g^{-1}$) and lower in the treatment triclopyr 0.75% e.a. (0.5850 $g.g^{-1}$) (Figure 3A). These values were found in a phase of great foliar growth, since it coincides with the period after the recovery of the intoxication of the plants, and the photoassimilates are allocated in the leaves.

Mean AGR or mean growth rate was 0.77; 0.78; 0.35; 0.57 and 0.32 g day^{-1} in the control and in treatments triclopyr 0.75% and, triclopyr 1.5% e.a., fluroxypyr + triclopyr 0.75 e. a. and fluroxypyr + triclopyr 1.5%, respectively in the 15 to 75 days after application.

Plants subjected to the treatments and 0.75% triclopyr, triclopyr and fluroxipyr + 0, 75% and remained similar to the control, and reached the maximum GRG

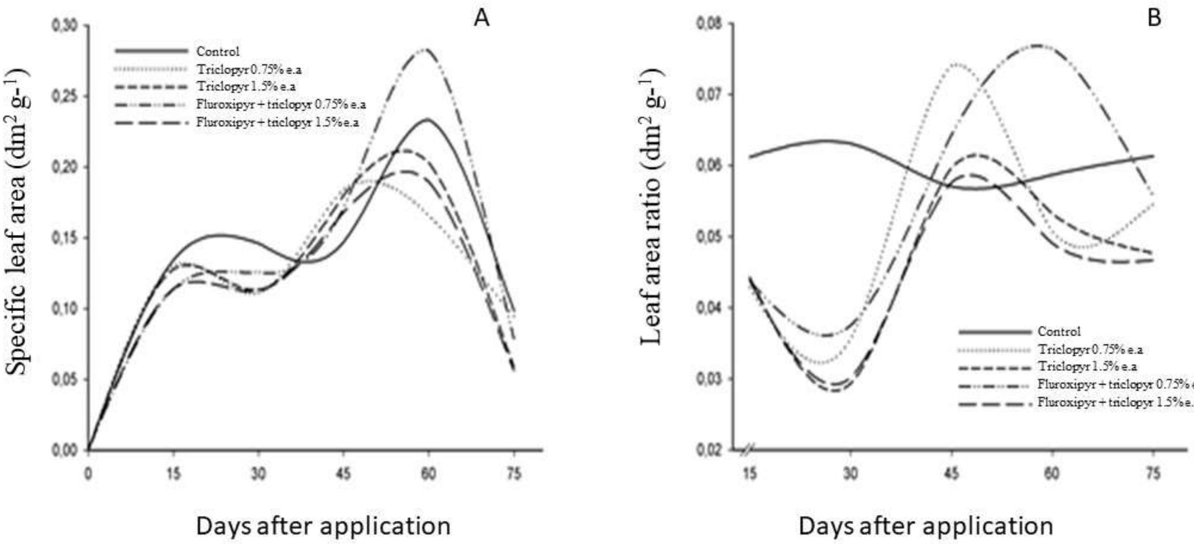

Figure 2 – Specific leaf area - SLA (A) and leaf area ratio - LAR (B) of the hybrid *E. urograndis* as a function of time, in response to the application of the herbicides triclopyr and fluroxipyr + triclopyr in two doses (e.a. – equivalente acid). Gurupi-TO, 2014.

Figura 2 *– Área foliar Específica - AFE (A) e Razão de área foliar - RAF (B) do híbrido **E. urograndis** em função do tempo, em resposta a aplicação dos herbicidas triclopyr e fluroxipyr+triclopyr em duas doses (e.a - equivalente ácido). Gurupi-TO, 2014.*

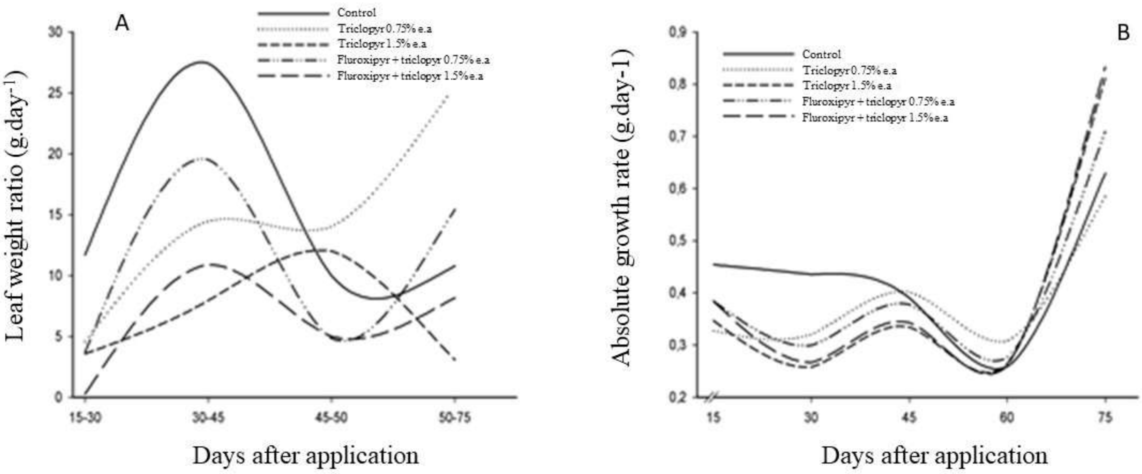

Figure 3 – Leaf weight ratio – LWR (A) and absolute growth rate – AGR (B) of *E. urograndis* hybrid in function of time, in response to application of the triclopyr and fluroxipyr + triclopyr herbicides in two doses (e.a. - equivalent acid). Gurupi - TO, 2014.

Figura 3 *– Razão de peso foliar - RPF (A) e Taxa de crescimento absoluto - TCA (B) do híbrido **E. urograndis** em função do tempo, em resposta a aplicação dos herbicidas triclopyr e fluroxipyr+triclopyr em duas doses (e.a. - equivalente ácido). Gurupi-TO, 2014.*

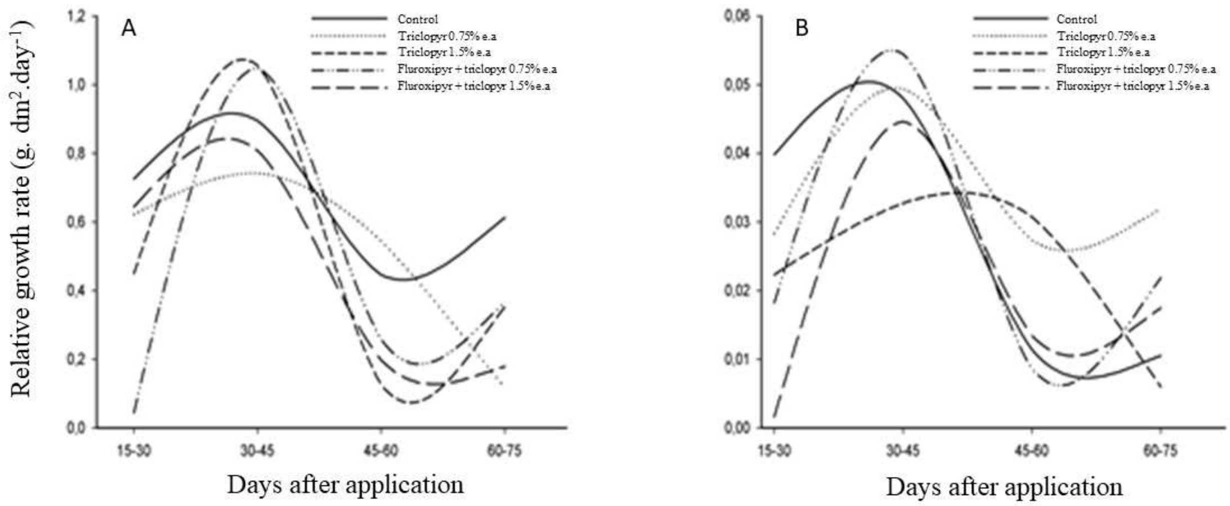

Figure 4 – Relative growth rate – RGR (A) and net assimilation rate - NAR (B) of *E. urograndis* hybrid in function of time, in response to application of the triclopyr and fluroxipyr + triclopyr herbicides in two doses (e.a. - equivalent acid). Gurupi -TO, 2014.

***Figura 4** – Taxa de crescimento relativo - TCR (A) e Taxa assimilatória líquida - TAL (B) do híbrido **E. urograndis** em função do tempo, em resposta a aplicação dos herbicidas triclopyr e fluroxipyr+triclopyr em duas doses (e.a - equivalente ácido). Gurupi-TO, 2014.*

0.0494 and 0.0545 $g.g^{-1}.day^{-1}$ respectively, from 30-45 DAA, whereas those that did not receive application of herbicides presented 0.0480 $g.g^{-1}dia^{-1}$ in the same period.

In the treatment of triclopyr 1.5% and a more pronounced effect was observed in the reduction of the GRG, and in the period of 30-45 DAA, all the treatments reached the maximum GRG, while the plants submitted to the treatment triclopyr 1.5% and maintained was stable in this period with evident reduction from 60 DAA (Figure 4A).

The maximum NAR values obtained were 1.0556 and 1.0473 $g\ cm^{-2}\ day^{-1}$ for the treatments triclopyr 1.5% e.a. and fluroxypyr + triclopyr 0.75% e.a in the period of 30-45 DAA, respectively. All treatments, including the control, after the period of 30-45 DAA, showed a sharp decrease in NAR. The increase in NAR in plants treated with auxin mimic herbicides in the period 30-45 DAA may have occurred due to the effect of these herbicides on plant growth, however, after this period the plants began to recover from the deleterious effects of the herbicides, producing smaller leaves and avoiding self-shadowing, providing high assimilation of CO_2 (Figure 4B).

4. DISCUSSION

It was verified that the herbicide triclopyr when applied at the highest dose results in a greater stimulus in leaf area reduction compared to the control, this effect was observed when the herbicide triclopyr was applied alone, as well as in a mixture formulated with the herbicide fluroxipyr.

High concentrations of synthetic auxins can induce in the plant the synthesis of ethylene, responsible for senescence, thus producing symptoms characteristic of epinephosis, wilting, chlorosis and leaf fall, which are side effects that occur through the action of this hormone, but which has paper important in the death of susceptible plants (Braga et al., 1999; Wei et al., 2000). The already developed and mature leaves were less affected by the synthetic auxins, since they have few meristematic tissues, place of action of these herbicides (Vidal, 1997). In young leaves, the vessel-bearing veins grow rapidly under the stimulus of an auxinic herbicide, whereas the internerval tissue does not follow the same growth rate, because it does not receive the same stimulus, so the leaf becomes wrinkled (Carlin et al., 1971). In some sensitive crops, such as tomato, grape and cotton, the young leaves characteristically deform, and this symptom is called "frog-leg" (Guevara, 1998).

The auxin mimetic herbicides act in the synthesis of the ethylene hormone, which in turn can inhibit cell expansion and act on leaf senescence, resulting in a decrease in SLA (Senseman, 2007).

The specific leaf area refers to the relationship between leaf area and dry leaf mass, considered as the morphological and anatomical component of LAR, because it relates the surface (leaf area, dm^2) to the dry mass of the leaf itself (g), indicating the leaf thickness (Cairo et al., 2008).

Increasing the dose of glyphosate (0-345.6 g e.a ha^{-1}), herbicide that also acts on the ethylene synthesis, promotes the increase in the area and the thickness of the palisade parenchyma, resulting in increased leaf thickness as a response of plants to compensate for loss of leaf area due to senescence (Santos et al., 2008).

At 75 DAA, both herbicide treated plants, both in the two doses, as well as the controls showed stabilization of the relationship between the specific leaf area and the weight of its leaves.

The leaf area ratio (LAR) is expressed as the area useful for photosynthesis and is a morphophysiological component, representing the leaf area in dm^2 that is being used by the plant to produce 1 g of dry matter (Benincasa, 2003).

For most crops LAR rapidly increases to a maximum in the vegetative period, stabilizing later, with the development of the crop. This response indicates that, initially, most of the photosynthesized material is converted into leaves, aiming at the greater capture of the available solar radiation (Pereira and Machado, 1987).

All treatments, including the control, showed a reduction of LWR in the period between 15 and 60 DAA, with a subsequent increase up to 75 DAA, due to the gain of foliar mass in this period, due to the detoxification of the plants.

The leaf weight ratio (LWR) is the ratio of dry matter to dry matter accumulated in the plant, which expresses the fraction of dry matter not exported to the rest of the plant (Benincasa, 2003).

It is common the tendency of LWR decrease, due to the export of photoassimilates from the leaves (sources organs) to other organs of the plant, that need to use them as substrates for its maintenance and growth. From a certain stage of growth, the increase in plant weight then occurs at a greater intensity than the increase in leaf weight, due to the formation of non photosynthetic organs such as branches, trunk and roots, and to the growing demand for photoassimilates. Thus, the proportion of leaf weight and plant weight becomes lower, which promotes a decline in LWR. This effect is verified under normal conditions, however under stress conditions (simulation of herbicide drift) the opposite effect can be observed (Cairo et al., 2008).

Alves (1998) found that the LWR of two eucalyptus species (*E. grandis* and *E. urophylla*) differed only in the evaluation of 120 days after sowing, when the value presented in *E. grandis* was higher in 18.6% to that of *E. urophylla*.

One of the fastest hormonal responses observed in plants is the induction of auxin growth. The growth response begins 10 minutes after the plant tissue has received auxin, resulting in growth rates that persist for several hours and, depending on the type of tissue or plant, can last for several days (Park, 1998; Wei et al., 2000).

The increase in the LWR may be associated, to a certain extent, with the increase of the leaf area and the amount of photoassimilates produced, with the purpose of plant growth and development (Lopes and Maestri, 1973).

AGR can be used to give an idea of the average growth velocity over the observation period (Benincasa, 2003).

The relative growth rate (RGR) represents the plant growth variation in a given time interval, taking into account the pre-existing values, prior to each variation, and is therefore an estimate of the plant's efficiency in accumulating dry matter (Benincasa, 2003).

The decrease in RGR with plant age results in part from the gradual increase of non photosynthetic tissues with plant development due to the increase in respiratory activity, variations in climatic conditions and abiotic effects, which can be evidenced by the increase in leaf area index (Lopes and Maestri, 1973; Benincasa,1988).

In the present study the net assimilation rate (NAR) between the analyzed periods had different behaviors among the treatments, so that the applied herbicides negatively affected the NAR in the hybrid *E. urograndis*, in all periods, mainly in the first and last period of analysis (15-30 and 60-75 DAA). It is known that NAR is a measure of the increase of dry mass through photosynthetic products (CO_2) of the plant per unit of leaf area, evidencing the efficiency of the photosynthetic apparatus. This characteristic varies more with the age of the plant than with the climatic factors (Benincasa, 2003).

The observed tendency is that the NAR is higher at the beginning of the crop cycle, which is explained by the lower auto-shading. It should be clear that the NAR is dependent on the size of the leaf area, leaf distribution in the canopy, leaf angle, translocation and assimilation partition (Gondim et al., 2008; Moraes et al., 2011).

5. CONCLUSIONS

The drift of herbicides triclopyr and fluroxypyr + triclopyr in the hybrid *E. urograndis* impairs the development of the plants at the beginning of field establishment. The dry matter accumulation mass of plants under the effect of the herbicide drift was not influenced until the 45 DAA, evidenced by the GRG. At 75 days after simulation of the herbicide drift, the plants present recovery characteristics of intoxication symptoms. It is necessary to perform studies superior to 75 DAA to prove the total recovery of intoxication symptoms in *E. urograndis* hybrids resulting from the herbicide drift under study.

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