TOLERANCE OF EUCALYPT (*Eucalyptus* spp.) SEEDLINGS TO IMAZAPYR IN NUTRITIVE SOLUTION¹

Tolerância de Mudas de Eucalipto (Eucalyptus spp.) ao Imazapyr, em Solução Nutritiva

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ABSTRACT - Imazapyr has presented excellent results in controlling coppices in stand reforms of eucalypt forests, despite differences in the efficacy levels. To find out whether these different responses are caused by the genetic variability of the cultivated materials, two experiments were carried out under greenhouse conditions with different imazapyr doses in a hydroponic system in plastic vases containing 2,500 mL solution. The clones IEF-1 (*Eucalyptus grandis* x *Eucalyptus* sp. hybrid), GE 463 (*E. urophylla* x *E. grandis*), and MN 445 (*E. grandis* x *Eucalyptus* sp. hybrid) were used in the first assay, and IEF-1, IEF-2 (*E. grandis* x *E. urophylla*) x *Eucalyptus* sp. hybrid) and the clones 129 and 7182 (*E. grandis* x *Eucalyptus* sp. hybrid) and the clones 129 and 7182 (*E. grandis* x *Eucalyptus* sp. hybrid) to the solution at doses of 0.00, 0.05, 0.10, 0.20, 0.40, 0.80, 1.60 and 3.20 μ L L⁻¹. Clone GE 463 proved to be more tolerant to imazapyr than clones IEF-1 and MN 445 in the first assay; however, in the second, clone 7182 was the most tolerant. Thus, doses should also be differentiated when controlling coppices, according to the cultivated clone.

Key words: Chopper NA, Eucalyptus grandis, E. urophylla, herbicide, dose-response.

RESUMO - O imazapyr tem apresentado excelentes resultados no controle de brotações na reforma de cultivos florestais com eucalipto, porém têm sido verificados diferentes níveis de eficiência. Com o intuito de averiguar se as diferentes respostas encontradas podem ser devido à variabilidade gênica dos materiais cultivados, foram realizados dois bioensaios em casa de vegetação, utilizando diferentes doses de imazapyr, sob sistema hidropônico (solução de Clark), em vasos plásticos contendo 2.500 ml de solução. No primeiro ensaio trabalhou-se com os clones IEF-1 (híbrido de Eucalyptus grandis x Eucalyptus sp.), GE 463 (E. urophylla x E. grandis) e MN 445 (híbrido de **E. grandis** x **Eucalyptus** sp.) e, no segundo , com IEF-1, IEF-2 (híbrido de (E. grandis x E. urophylla) x Eucalyptus sp.) e os clones 129 e 7182 (híbridos de E. grandis x Eucalyptus sp.). Trinta dias após o transplantio das mudas para solução nutritiva, fez-se a aplicação de imazapyr à solução nas doses de 0,00; 0,05; 0,10; 0,20; 0,40; 0,80; 1,60; e $3,20 \ \mu L \ L^{-1}$. O clone GE 463 mostrou-se mais tolerante ao imazapyr que os clones IEF-1 e MN 445, no primeiro ensaio; já no segundo, o clone 7182 foi mais tolerante. Os clones apresentaram comportamento distinto entre si quanto à presença de imazapyr em solução, indicando que para o controle de rebrota as doses também podem ser diferenciadas, de acordo com o clone cultivado.

Palavras-chave: Chopper NA, Eucalyptus grandis, E. urophylla, herbicida, dose-resposta.

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INTRODUCTION

Eucalypt (*Eucalyptus* sp.) was introduced in Brasil in 1903, for commercial purposes by Edmundo Navarro de Andrade at Companhia Paulista de Estrada de Ferro, to supply firewood for the production of ties and telephone poles, due to its adaptability to different climate, soil, and altitude conditions of the state of São Paulo (Filgueiras, 1989). Nowadays, worldwide plantation areas of eucalypt are estimated to exceed 15 million ha, of which approximately 40% lie in Brazil, where this genus is the most commonly used for reforestation, with distinct economic purposes (SBS, 2003).

In recent years, Brazil has become a great exporter of forest products, on account of its great competitiveness in this field (Silva et al., 1997), and reforestation has become an excellent investment alternative. According to data of the Sociedade Brasileira de Silvicultura (SBS, 2003), reforested areas in Brazil currently amount to 5.0 millions of hectares.

Due to the great demand for exportation and domestic market of paper, cellulose, charcoal, and, more recently, sawmill industries (for furniture making), vast reforested areas need to be maintained at low cost, without affecting forest production, wood quality, or the environment.

One of the most common problems forest sector companies have to tackle with is deciding the most viable alternative for the future conduct of the stand, i.e., wether to renew the present crop or not (Simões, 1981). If the choice is to reform, coppices will have to be controlled to avoid any competitive effect on newly-planted seedlings (in the space between the previous crop rows) (Little et al., 1996). It is preferable to kill stumps before replanting commences as the rapid initial growth of the coppice would require earlier control than for normal weeding operations. The height of the coppice regrowth would require nozzles to be raised during herbicide spraying, thus increasing the risk of seedling damage through spray drift (Morze, 1971). Some of the methods used to control coppicing are: fire (Poggiani et al., 1983); manual

removal of the stump's outer bark; grade "bedding", which also marks planting and fertilization rows (Simões, 1981; Ripasa, 1983); mechanized stump extraction (Vieira & Da Silva, 1983; Ripasa, 1983; Moro et al., 1988; Guerreiro et al., 1990); and herbicide application over cut surface immediately after felling of the trees (Little & Eccles, 2000). However, according to Christoffoleti et al. (1997), imazapyr can be applied up to 72 hours after felling with high control efficacy. Another form of coppice control without spray drift risks is injecting imazapyr in the tree trunk (Respondovesk, 1999), besides the different efficacy levels have been observed in coppice control after felling the trees.

According to Whitford et al. (1995), coppice control efficacy depends on the species, herbicide, dose, spacing of the injections and season; besides vapor pressure deficit and soil water deficit on the day before application of the herbicide are important. The authors recommend that for stem injection of E. marginata trees, glyphosate at a dose of 20 g a.i. m⁻² of stem basal area be used in winter, spring and autumn, while for *E. calophylla*, 80 g a.i. m^{-2} of glyphosate is recommended for winter and autumn, provided that, for both species to achieve high mortality, vapor pressure deficit on the day prior to stem injection is less than 1.5 kPa. Dantas et al. (2001) verified differences in the coppice control of eucalypt clones after insertion and injection of glyphosate into the tree, with nearly 55% efficacy 90 days after cutting. In species that tolerate "over the top" application (foliar contact of the herbicide), such as some conifers, selectivity is greater in wintertime, when the plant does not present visible growth, i.e., during its dormancy period (Ahrens, 1981) - period in which trees decrease their physiological activities due to unfavorable conditions. Campbell & Nicol (1998), working with tolerance of tree seedlings to herbicide applied in pre- or post-emergence, noted that Pinus radiata was more tolerant to herbicides in witch Eucalyptus, Acacia and Casuarina species, since P. radiata tolerated 0.36 kg ha⁻¹ a.i. (glyphosate) while other species could only tolerate 0.18 or 0.09 kg ha⁻¹ a.i. However, some eucalypt species were more tolerant to glyphosate than

Tolerance of eucalypt (Eucalyptus spp.) seedlings ...

others, e.g., *E. viminalis* stood to 0.18 kg ha⁻¹ a.i. without toxicity symptoms, with the same being verified to *E. globulus*, *E. mannifera* and *E. viminalis*, when applied 0.09 kg ha⁻¹ a.i.

Relative humidity, light intensity, temperature, and physiological activities, such as photosynthate translocation and xylem pressure potential, can influence herbicide uptake and translocation (Radosevich & Bayer, 1979; King & Radosevich, 1985), giving rise to variations in the response of each species to herbicide applications (Gratkowski, 1977; Radosevich et al., 1980; Paley & Radosevich, 1984).

The selectivity mechanism of imazapyr in some species appears to be a consequence of differences in uptake and translocation, and molecule metabolization in relatively immobile and non-toxic compounds, modifying its half-life in the plant (Shaner & Mallipudi, 1991). Still, in the case of eucalypt, other factors likely to interfere with the herbicide tolerance have not yet been studied, since the license for using this molecule for coppice control in Brazil has only recently been obtained (Cyanamid, 1999).

Thus, the aim of this work was to evaluate the tolerance of *Eucalyptus* clones to imazapyr.

MATERIAL AND METHODS

Two assays were carried out under greenhouse conditions, using different doses of imazapyr (0.00, 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, and 3.20 μ L L⁻¹), which were mixed with the nutritive Clark solution (Clark, 1975) in plastic vases holding 2,500 ml of the solution. The pH was corrected every other day to 5.50 ± 0.10 with NaOH or HCl. The vases were painted with silver color on the outer side, and with a double plastic layer, the first black and the other transparent, on the inner side. The vase lids were perforated with holes of 14 and 2 mm; the largest was used to insert the seedlings, and smallest hole to pass an air tube, connected to a compressor with constant airflow, into the solution.

In the first assay, non-commercial seedlings of IEF-1 (*E. grandis* x *Eucalyptus* sp. hybrid), and the clones GE 463 (*E. urophylla* x *E. grandis*) and MN 445 (*E. grandis* x



Eucalyptus sp.) were used. The seedlings were 4-months old and 20 to 25 cm high, cultivated in a substrate of vermiculite and carbonized rice chaff, in stiff plastic tubes of 55 cm³. Homogeneous seedlings were used for replanting, after washing the root system, whereupon they were immediately transferred

carbonized rice chaff, in stiff plastic tubes of 55 cm³. Homogeneous seedlings were used for replanting, after washing the root system, whereupon they were immediately transferred to the hydroponic vases. Pipettes applied the herbicide doses 30 days after the seedling transplant. Forty days after the treatment application (DAT), eucalypt toxicity was evaluated by the symptomatic scale of the European Weed Research Council (EWRC, 1964), which attributes grades 1 to 9, representing toxicities of 0, 1-15, 16-30, 31-45, 46-60, 61-75, 76-90, 91-99, and 100%, respectively, where 0 (zero) is the absence of symptoms and 100% means plant death. Height and dry biomass of the aerial part (DBAP) and roots (DBR) were also evaluated. After separating the aerial part from the roots, these were separately placed in paper bags, placed in a stove with air circulation, and maintained at a temperature of 72 ± 1 °C until weight became constant (after approximately 72 hours), for dry biomass determination. The factorial scheme was $3 \ge 8$, with three clones and eight herbicide doses.

In the second assay, the same methods described for the first were used, but with a modified hybrid composition: clones 129 and 7182 (*E. grandis* x *Eucalyptus* sp. hybrids), and IEF-1 and IEF-2 ((*E. urophylla* x *E. grandis*) x *Eucalyptus* sp. hybrid). Seedlings were three months and a half old and 15 to 20 cm of hight. The factorial scheme was 4×8 , with four clones and eight herbicide doses.

A completely randomized statistical design was used, with eight treatments corresponding to the herbicide doses, in four replications. The obtained data were submitted to the analysis of variance (ANOVA) after verifying the presuppositions (experimental errors must have a normal distribution, a common variance and must not be correlated among them, and the model parameters must be additive) (Demétrio, 1978). After ANOVA (Table 1), a regression analysis was carried out using the log-logistic model to evaluate the dose-response to height (assay 1) and DBR, and logistic model to DBAP and height (assay 2). The statistical software package

Source of variation	DE	Mean squares		
	D.F.	PH	DBAP	DBR
First Assay				
Genotype	2	26,35042 ^{ns}	28,74095**	4,978517**
Treatment	7	830,9778**	537,0937**	29,19644**
Genotype x Treatment	14	65,08412**	33,10273**	2,030421**
Residual	72	11,47459	2,441989	0,2889355
Coef. of variation	-	7,505	13,166	16,696
Second Assay				
Genotype	3	945,1536**	0,7620196**	0,3428448**
Treatment	7	1.163,775**	1,322194**	1,178601**
Genotype x Treatment	21	62,30839**	0,07007759**	0,08063768**
Residual	96	4,736918	0,008144170	0,008695483
Coef. of variation	-	8,153	22,0972	33,875

Table 1 - Summary of analysis of variance for plant height (PH), dry biomass of aerial part (DBAP) and dry biomass of roots (DBR) of eucalypt plants under imazapyr doses, in a hydroponic system

ns: denotes non-significance by F-test at 10% of probability; **: denotes significance by F-test at 1% of probability.

Saeg (SAEG, 1993) was run for the analysis of variance, and the SigmaPlot program (SIGMAPLOT, 1997) for the parameter estimation of the regression model.

The adopted log-logistic model – equation 1 (Seefeldt et al., 1995) – has four parameters, where y_0 is the inferior limit, i.e., the mean response at the highest dose; *a* is the difference between the superior and the inferior limit, i.e., the evaluated characteristic amplitude between the highest and the lowest dose; and *b* describes the slope of the curve around I₅₀ (dose with a response of 50% of inhibition), given by the value x_0 .

$$y = y_0 + \frac{a}{1 + \left(\frac{x}{y_0}\right)}$$
 (eq. 1)

The logistic model – equation 2 (Finney, 1979; Streibig, 1988; Souza et al., 2000) – has three parameters as follows: *a* is denominated "saturation level", corresponding to the bio-indicator response at the lowest dose; x_0 is the inflection point of the curve, which corresponds to the value of I_{50} ; and *b* is the slope of the curve around I_{50} .

$$y = \frac{a}{1 + \left(\frac{x}{y_0}\right)^b}$$
 (eq. 2)

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RESULTS AND DISCUSSION

Clone MN 445 presented a height of 66.7 cm, superior to the IEF-1 with 58.9 cm and GE 463 with 52.6 cm, in the treatment without herbicide application (Figure 2). However, MN 445 and GE 463, at doses above 0.8 μ L L⁻¹ a.i., presented similar heights (y_0 = 39.3554 and 38.0200, respectively), about 25% superior to IEF-1 (y_0 = 29.3688), and, therefore, less sensitive to higher herbicide doses. Thus, hybrid IEF-1 was the most sensitive to imazapyr as the concentrations increased. This fact was observed by the lower plant height of this clone, together with high toxicity, obtaining grades 8 and 9 for the doses 1.6 and 3.2 μ L L⁻¹ a.i., respectively (Figure 1B).

Clones IEF-1 and GE 463 presented a similar I_{50} ($x_0 = 0.2515$ and 0.2548, respectively), about 75% above the value found for clone MN 445 ($x_0 = 0.0669$). This means that the dose-effect response in I_{50} was much more intense in this last one, presenting a higher sensitivity to small increments at doses below $0.4 \ \mu L \ L^{-1}$ a.i. (Figure 2).

The smallest amplitude between the highest and lowest doses (*a*), the smallest slope of curve-response (*b*) and the highest I_{50} value (x_0) showed that clone GE 463 was the most tolerant to increased herbicide doses, since it presented a smaller effect on the height in relation to clones IEF-1 and MN 445.





Figure 1 – Percent and grades of toxicity of the eucalypt plants (A – first assay and B – second assay) in relation to imazapyr doses at 40 days after treatment application.



Figure 2 - Dose-response curve of plant height of IEF-1 (●), GE 463 (■), and MN 445 (▼) clones, under increasing doses of imazapyr.

For the dry biomass of the aerial part (Figure 3), clone GE 463 presented an I_{50} of 0.4603 µL L⁻¹ a.i., 63.9% superior to IEF-1 (with 0.1663 µL L⁻¹ a.i.), and 80.8% superior to MN 445 (with 0.0886 µL L⁻¹ a.i.). The latter was more sensitive to small increments of

doses up to 0.2 μ L L⁻¹ a.i. Increased imazapyr concentrations above 1.6 μ L L⁻¹ a.i. did not interfere with the DBAP growth of clones, since above this dose the plants died (grade 9). Clones GE 463, IEF-1, and MN 445 presented a DBAP of 20.8, 25.3 and 26.4 g per vase,



respectively, in the treatment without herbicide application.

The values of the curve slopes (*b*) around the dose that inhibited 50% of the aerial part growth indicated a softer slope for clone GE 463, since the tangent line to the curve at the I_{50} value presents a greater angle with the axis of the imazapyr doses, thus expressing, together with the I_{50} value, a greater tolerance of this clone to imazapyr (Figure 3).

Clones GE 463 and MN 445 presented similar behaviors of the dry root biomass (Figure 4) in relation to the control (zero dose) as well as to doses above $0.8 \,\mu L \,L^{-1}$ a.i. However, the first presented an I₅₀ (0.6065 $\mu L \,L^{-1}$ a.i.) of about 100% above the value found for IEF-1 (0.3186 $\mu L \,L^{-1}$ a.i.) and MN 445 (0.3657 $\mu L \,L^{-1}$ a.i.), which expressed a greater imazapyr tolerance of GE 463 compared to the others.

The clones used in the second assay presented similar heights (Figure 5) in the absence of imazapyr, but clones IEF-1 and IEF-2 presented a reduced growth in height as the herbicide concentrations increased, with the effect being more evident for the IEF-2 hybrid, because it presented a smaller I_{50} value ($x_0 = 0.1180$). Clones 7182 and 129 were more tolerant to imazapyr since the applied doses were insufficient to determine I_{50} , besides obtaining less grades of toxicity than the IEF-1 and IEF-2 clones (Figure 1A).

It was necessary to transform the DBAP and DBR by the equation $x_t = Log x$ (where x is the studied variable and x_t the transformed one) to obtain a normal data distribution.

The Log of the dry biomass yield of the aerial part (Figure 6) was similar to the clones IEF-1 (with 0.9816) and 7182 (with 0.9528) in the herbicide absence; however, the I₅₀ value was about three times higher for 7182 ($x_0 = 0.6043$) in relation to IEF-1 ($x_0 = 0.2384$), displaying a greater tolerance of this clone at doses below 0.8 µL L⁻¹ a.i. The superiority of this clone was also verified, for the same reasons, when compared to the I₅₀ value of IEF-2 ($x_0 = 0.1149$) and 129 ($x_0 = 0.0756$), since it



Figure 3 - Dose-response curve of dry biomass of aerial part of IEF-1 (●), GE 463 (■), and MN 445 (▼) clones, under increasing doses of imazapyr.



Tolerance of eucalypt (Eucalyptus spp.) seedlings ...



Figure 4 - Dose-response curve of dry biomass of roots of IEF-1 (●), GE 463 (■), and MN 445 (▼) clones, under increasing doses of imazapyr.



Figure 5 – Dose-response curve of plant height of IEF-1 (●), IEF-2 (▲), 129 (♦) and 7182 (◊) clones, under increasing doses of imazapyr.



was five times greater than the first and eight times greater than the second. These two last clones, besides presenting a smaller DBAP under imazapyr absence and smaller I_{50} values, also presented greater effect of the DBAP dose-response at doses above 0.8 μ L L⁻¹ a.i.

After analyzing the Log of the dry root biomass (DBR), it was verified that clone 7182 also showed more tolerance to imazapyr at doses below $0.4 \,\mu$ L L⁻¹ a.i., presenting a greater I₅₀ value among the hybrids, because increases at doses above this reduced the DBAP growth for the clones IEF-1 and 7182 (Figure 7).

The clones GE 463 and 7182 own some detoxification mechanism for the imazapyr molecule, which might involve oxidation, reduction, and/or, herbicide hydrolysis (Shaner & Mallipudi, 1991), or else, a smaller uptake amount of the molecule present in solution, which could have caused increased tolerance of these clones to imazapyr, compared to the others. Green et al. (1992) verified differences of glyphosate uptake and translocation when studying four forest species: loblolly pine (*Pinus taeda* L.) and yaupon (*Ilex vomitaria* (L.) Ait.) were more tolerant to this herbicide than to red maple (*Acer rubrum* L.) and white oak (*Quercus alba*) due to a lower molecule uptake. Moreover, *Q. alba* was more tolerant than *A. rubrum*, due to its ability to accumulate greater quantities of glyphosate in the roots.

Under the present experimental conditions, our results allow the following conclusions: the estimate of the I_{50} value varies according to the evaluated characteristic; and the efficacy of imazapyr used in eucalypt to control coppices may vary according to the clone used, since clones GE 463 and 7182 were more tolerant to imazapyr compared to the others.

The selection of more tolerant clones is very interesting when it is necessary to make chemical weed control after planting eucalypt seedlings, since the herbicide would control the weeds without killing the seedlings. However, when opting for sprouting control after cut-stumps, the use of high doses of herbicide is required or less tolerant clones must be used at planting.



Figure 6 – Dose-response curve of Log of dry aerial part biomass (DBAP) of IEF-1 (●), IEF-2 (▲), 129 (♦), and 7182 (◊) clones, under increasing doses of imazapyr.





Figure 7 - Dose-response curve of Log of dry biomass of roots (DBR) of IEF-1 (●), IEF-2 (▲), 129 (♦), and 7182 (◊) clones, under increasing doses of imazapyr.

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606